台灣省農業試驗所特刊第67號

中美農業科學合作研究計畫檢討會專刊

Proceedings of The Symposium for Reviewing COA-USDA Agricultural Science Cooperative Program

時間：中華民國八十六年五月五～六日
地點：台灣省農業試驗所　國際會議廳

古德業、葉瑩、林俊義　策劃
張清安、陳金枝　編輯

行政院農業委員會　主辦
臺灣省農業試驗所　協辦
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中華民國八十六年十二月

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中美農業科學合作研究計劃檢討會
Symposium for reviewing COA-USDA agricultural science cooperative program

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中美農業科學合作研究計畫檢討會程序表

時間：中華民國八十六年五月五日～六日
地點：台灣省農業試驗所　國際會議廳
台中縣霧峰鄉中正路189號

5月5日

08:00-09:00　報到Registration
09:00-09:40　開幕致詞 Opening remarks　邱主委，古處長(農委會，COA)
　來賓致詞 Guest speech　AIT representatives
　致歡迎詞 Welcome speech　林俊義所長(農試所，TARI)
09:40-10:00　團體紀念照及休息　Group Photo and Break

Session－漁業領域 (Fishery)
主　持　人：郭光雄
Co-presiding：Wallis H. Clark, Jr.

10:00-10:50　國外專家　Wallis H. Clark, Jr.
10:50-11:20　合作計畫綜合評述　陳秀男
10:20-11:45　分項重點　吳金洌　傳染性胰臟壞死病毒之分子生物學研究
10:45-12:10　分項重點　郭欽明　台灣養殖蝦類生理研究

12:10-13:30　午餐及休息 Lunch and Break

Session－林業領域 (Forestry)
主　持　人：楊政川
Co-presiding：S. Joseph Chang

13:30-14:20　國外專家　S. Joseph Chang
14:20-14:40　休息　Break
14:40-15:10　合作計畫綜合評述　洪富文
15:10-15:35　分項重點　任億安　美國木材在台灣使用狀況之剖析
15:35-16:00　分項重點　鄒哲宗　研發對環境安全且有效之住宅與商業用木製品抗燃劑

16:00-17:00　生技合作專題座談 Panel Discussion on Biotechnology　古德業
17:30-20:00　晚宴　Dinner

5月6日

Session－農業領域 (Agriculture)

主　持　人：林俊義

Co-presiding：Dennis Gonsalves

08:30-09:20　國外專家　Dennis Gonsalves

09:20-09:50　合作計畫綜合評述　劉顯達

09:50-10:15　分項重點　蘇鴻基　柑桔病原檢驗試劑與技術之開發與應用

10:15-10:45　休息　Break

10:45-11:10　分項重點　曾浩洋　食品病原菌檢驗技術之開發與應用

11:10-11:35　分項重點　張清安　重要植物病原檢測試劑與技術之開發與應用

11:35-12:00　分項重點　葉錫東　生物技術於病害防治上之應用

12:10-13:30　午餐及休息 Lunch and Break

Session－牧業領域 (Livestock)

主　持　人：朱瑞民

Co-presiding：Prem S. Paul

13:30-14:20　國外專家　Prem S. Paul

14:20-14:50　合作計畫綜合評述　張天傑

14:50-15:10　休息　Break

15:10-15:35　分項重點　黃政齊　熱帶與溫帶牛種在本省雜交肉用之研究

15:35-16:00　分項重點　潘銘正　構築以豬假性狂犬病病毒為載體之多價疫苗

Session－綜合討論 (General Discussion)

16:00-17:00　主　持　人：古德業　處長

葉　瑩　科長

18:00-20:00　歡送晚宴　Farewell Party

中美農業科學合作計畫綱領推動情形報告

行政院農業委員會　古德業

一、中美農業科學合作計畫綱領簽署緣起

民國七十四年二月底，李總統在副總統任內曾率團(包括本會葛副主委錦昭)赴南美烏拉圭訪問，途經美國紐約市，並與美國在台協會丁大衛主任會晤，談及運用我國現有之部分中美社經基金辦理雙邊農業技術合作計畫。當時，李副總統甚表贊同。同年三月十二日美國在台協會蓋哲甫(Joseph. B. Kyle)先生致函前北美事務協調委員會(CCNAA)駐美辦事處，告知美方擬與我簽訂農業科技合作協定事宜。CCNAA駐美辦事處旋於三月廿一日呈報外交部並副知本會。同時該處並安排美國農部專家Jerry S. Walker博士於同年三月間來華到本會商討初期合作研究內容，雙方對柑桔病害、豬病、森林、養殖漁業、食品加工等方面具有合作研究興趣。

有關中美擬進行農業科技合作，本會於七十四年四月十日函外交部敬表贊同，外交部於五月廿四日函送中美「農業科學合作計畫綱領」草案內容至本會，本會乃於六月十七日函復該部對協定內容之修訂意見，並建議協定書美方執行單位為美國農業部，我方執行單位由農委會負責，以建立雙方官方管道實質合作基礎。其後，由外交部責成CCNAA將我方綜合修正意見答覆美方，並經美方同意於七十五年一月廿八日正式完成簽署換文，實際工作之推動由本會及美國農部負責。

二、合作計畫綱領內容摘要

㈠目的：

1.為增進中美雙方在農業科學家間與農業研究暨高等教育機構間之聯繫與合作；

2.為提供研究人員與研究機構交換資料、觀念及技術之機會；

3.為增進合作解決共同關切問題之機會；以及

4.運用特殊設備或現有之研究機會。

㈡合作範圍及方式：

本合作計畫綱領之合作，除得在基礎與應用科學之任一擴大範圍中進行外，並包括林業與農業經濟學。合作推動之方式則包括科學資料交換、專家互訪、學術與實務研討會，以及合作研究。

㈢經費籌措：

本合作計畫綱領項下合作活動之經費籌措(中方由農委會，美方由美國農部)原係在相對、互惠與彈性之基礎上進行，由雙方各自負擔己方參加合作活動之費用，亦得依其選擇為彼方參加此項活動之科學家負責部分或全部費用，而共同活動之經費籌措則由雙方協議定之。開始時雙方逐年各編列年度預算一百萬美元等值支應。唯後來美方經費短絀，自一九九二年起僅能提供差旅費。

(四)合作內容：

1.著重新作物栽培及病蟲害防治的生物控制技術。

2.保育資源與維持或增加產量並行的新生產方法。有效使用土壤、水分與空氣資源的系統。

3.具有顯著農林業價值的動植物與微生物種原。

4.應用於動植物生殖與保護的基礎研究與生物科技。

5.收穫後處理技術。

6.養殖漁業

其他合作內容項目由本會與美國農部研商同意後，隨時加列。

三、擴大合作計畫綱領續簽項目內容

(一)中美「農業科學合作計畫綱領」自七十五年一月間簽署以來，雙方已進行九期計六十一項合作研究計畫。研究內容包括養殖漁業、植物病蟲害、林業、畜產及動物疾病、食品安全及加工技術、收穫後處理及設施栽培等。其中除第一期七項合作研究計畫經費全由美方支援外，二—五期之合作研究計畫經費則由雙方洽商同意分項支援，美方並對合作進展，表示滿意。七十七年九月間美國農業部代表Dr. Jerry S. Walker 來華洽商第三期合作有關細節及經費預算時，即表示未來希望增加有關合作項目，同意在原合作計畫綱領六項合作內容外，增列(1)農產運銷與發展；(2)森林經營；(3)自然生態及野生動物保護研究與專業人才培育；以及(4)農業、林業、農地保護、土地規劃及都市發展等專業人才培育與訓練等四項內容。該項續簽協定換函手續業由前北美事務協調委員會與美國在台協會代表中美雙方於七十八年九月十一日簽署完竣。

(二)另在「中美農業科學合作計畫綱領」架構下，於八十年(一九九一)二月與美國農業部經濟研究署簽署「重要農產品產銷分析與預測計畫」備忘錄，辦理主要農產品產銷分析與預測研究；於八十年六月與美國農業部林務署簽署「中美林業與自然資源保育合作備忘錄」，加強雙方林業與自然保育之科技研究合作以及人才培育與交流；以及於八十一年十月與美國農業部水土保持署簽署「中美水土保持科技交流與合作

備忘錄」，加強雙方水土保持技術交流、研究合作及人才培育等。

㈢一九九二年美方同意進行「食品監測技術」及「香菇生產技術」等兩項計畫，一九九二年中美雙方於本年三月商洽同意再進行十一項計畫，但未如往年一樣進行簽約，因美方無經費配合，僅能提供國外旅費。

四、執行概況

㈠第一期(一九八六)合作計畫

依據本合作畫之內容，第一期(一九八六)我方經由本會策劃研提九項合作計畫於七十五年四月底送美方審核，結果有七項計畫被接受，包括養殖漁業、植物病理、林業及獸醫等研究項目，美方代表Dr. Jerry S. Walker 並於七十五年九月來華兩週，商討有關合作細節及美方經費撥款事宜，同年九月，雙方審定第一期七項合作計畫如表一。

表一、中美農業科技第一期(一九八六)合作計畫項目

計畫編號	計　畫　名　稱	執　行　機　構	主持人	期限
TW-FS-11	菌根菌之接種與礦區廢土石地之復舊造林	台灣省林業試驗所	鍾旭和	四年
TW-ARS-6	台灣家禽流行性感冒監測	中興大學農學院 台灣省家衛所	謝快樂 呂榮修	三年
TW-AES-3	草蝦生殖生理與成熟控制研究	台灣大學漁科所	郭欽明	三年
TW-AES-8	噬菌體誘導(致)柑桔潰瘍病菌遺傳性質之變化及其對致病力之影響	中興大學農學院	吳文川	三年
TW-AES-7	台灣豬假性狂犬病控制與流行病學研究	中興大學農學院	王吉德	五年
TW-AES-4	水車對漁池影響之研究	中山大學	方新疇 方力行	三年
TW-AES-1	高成長率吳郭魚育種之研究	省水試所東港分所	黃齊明	五年

第一期七項合作計畫經費全由美方負擔，合計美金一百萬元(新台幣三五、八五二、八〇〇元)，已依計畫執行概況，及經費分配額，每半年由美方撥款一次，依約撥付計畫執行單位，並由本會負責會計及稽核作業。七項計畫已執行完成，美方對合作進展，極為滿意。

㈡第二期(一九八七)合作計畫

1.本會於七十五年七月間召集有關農業學術及研究機構等機關首長，以及第一期合

作計畫主持人開會，除報告第一期合作計畫外，並請各有關單位踴躍準備研提第二期(一九八七)合作計畫。美方代表Dr. Jerry S. Walker 於七十五年九月間來華兩週，洽商中美雙方第二期合作研究計畫項目。合作範圍商定包括養殖漁業、林業、畜產及動物疾病、食品安全、收穫後處理及植物病理等。本會函請有關單位研提合作計畫送美國農業部複審。，共十三項合作計畫，美方同意推動。

2.為因應往後雙方合作計畫之有效推動，並依據合作綱領之協定，經費負擔原則，本會自七十六(一九八七)年度起相對編列一百萬美元，以資配合。

3.七十六年八月初美方代表共計七人來華商談第二期(一九八七)合作計畫之執行，並與本會洽商雙方同意進行之十三項合作計畫細節。總經費預算為五五、八六〇、〇〇〇元，其中美方支援二九、二五〇、〇〇〇元，我方支援二六、六一〇、〇〇〇元。美方主要支援預算項目為人事費、津貼及國外差旅費；我方支援項目包括材料費、勞務費、國內差旅費、設備費及雜費等。第二期合作計畫項目如表二

表二、中美農業科技第二期(一九八七)合作計畫項目

計畫編號	計　畫　名　稱	執　行　機　構	主持人	期限
TW-ARS-9	大豆油耐高溫穩定性研究	食研所	朱燕華	四年
TW-ARS-10	降低果實貯藏寒害之研究	台灣大學農學院	王自存	四年
TW-ARS-11	少量有機添加物防治*Rhizoctonia solani*苗猝倒病之研究	台灣大學農學院	莊再揚	三年
TW-ARS-12	建立甘藷病毒標準檢定制度	亞蔬中心 中興大學	徐堯煇	四年
TW-AES-13	養殖蝦類傳染性病之研究	台灣大學理學院	郭光雄 陳秀男	四年
TW-AES-14	養殖草蝦之蛋白質需求與消化研究	台灣海洋學院	孫寶年	五年
TW-AES-15	純種及雜交種肉牛遺傳與環境相關性之研究	省畜試所恆春分所	洪嘉謨	五年
TW-AES-17	豬弓蟲病之研究—疫學、病理學及疫苗開發	屏東農專	張甘楠	五年
TW-AES-18	豬附紅血球立克次體病—診斷、免疫及防治之研究	台糖畜研所	徐興鎔	三年
TW-FS-12	美國木材在台灣之市場調查	省林試所	任憶安	二年
TW-FS-13	高比重闊葉樹材之加工研究	省林試所	唐讓雷	四年
TW-FSIS-1	生物快速測定系統發展	中興大學農學院	曾浩洋	四年
TW-FDA-1	輻射線對草蝦超微細結構及細胞化學之影響	食研所	楊瑞森	四年

(三)第三期(一九八八)合作計畫

本會於七十七年元月提供十三項合作研究計畫書至美國農部複審，結果共有八項合作計畫美方同意推動，主要合作範圍包括植物病理、養殖漁業、食品安全、獸醫及設施栽培。美國農部代表 Dr. Jerry S. Walker 和 Mrs. Gloria Yanosky 並於九月七日抵華洽商合作研究計畫有關事宜，經雙方商洽同意進行八項合作計畫，總經費預算四三、九九六、〇四〇元，其中美方支援一八、四九五、〇四〇元，我方支援二五、五〇一、〇〇〇元，美方主要支援項目為人事費、津貼及國外差旅費，我方支援項目為材料費、勞務費、國內差旅費、設備費及雜費等。本期合作項目如表三。

表三、中美農業科技第三期(一九八八)合作計畫項目

計畫編號	計畫名稱	執行機構	主持人	期限
TW-AES-27	草蝦之品質維護與廢棄物之利用	台灣海洋學院	陳幸臣	四年
TW-AES-24	吳郭魚耐寒性之生理生化與育種研究	台灣海洋學院 台灣省水試所	張清風 湯宏吉	四年
TW-AES-22	臺灣設施栽培系統之研究開發	農機發展中心 臺灣大學農機系	吳中興	三年
TW-FSIS-2	烹調與加工對肉品中殘留農藥及藥物之影響	台灣大學食科所	許順堯	三年
TW-FSIS-3	輻射照射對雞肉之影響	台灣大學食科所	郝龍斌	三年
TW-ARS-20	血清學探針之開發用於柑桔病原之檢驗與標誌探針應用於探測病原感染及傳播	台灣大學農學院	蘇鴻基	三年
TW-AES-19	傳染性鼻氣管炎自然感染牛與免疫牛鑑別診斷之研究	中興大學農學院	吳福明	四年
TW-AES-21	臺灣牛小型焦蟲之疫學研究及防疫	臺灣大學農學院	張政宏	四年

(四)第四期(一九八九)合作計畫

本會於七十八年二月初提送十一項合作研究計畫書至美國農部複審，結果共有十項計畫美方同意進行合作，其中七項計畫列入七十九年度(一九八九)執行、三項列入八十年度(一九九〇)再執行、及一項刪除。列入七十九年度執行之七項合作研究計畫，主要研究範圍包括植物病理、豬病及生理研究、魚病及疫苗開發研究等。美國農部代表Dr. Valdis E. Mezainis於九月間來華與本會洽商有關合作事宜。經

雙方洽商結果，同意七項合作研究計畫總經費預算為三八、八六二、〇〇〇元，其中美方主要支援一八、七七六、〇〇〇元，我方支援二〇、〇八六、〇〇〇元。本期合作計畫項目詳如表四。

表四、中美農業科技第四期(一九八九)合作計畫項目

計畫編號	計　　畫　　名　　稱	執　行　機　構	主持人	期限
TW-AES-31	台灣作物根部疫病之抑病土壤檢定及抑病機制研究	省農試所嘉義分所	安寶貞	四年
TW-AES-32	觀賞植物病毒快速檢測及其防治	中興大學植病系 臺灣省農業試驗所	柯南靖 張清安	四年
TW-AES-36	木瓜輪點病毒基因用在轉型木瓜植物之表現	中興大學植病系	葉錫東	四年
TW-DOI-1	養殖青魚死亡原因之研究	台灣大學理學院 省水試所	陳秀男 廖一久	三年
TW-AES-37	草蝦桿狀病毒診斷技術開發研究	中研院動物所 屏東農專獸醫科	吳金洌 董明澄	四年
TW-ARS-33	Lactoferrin基因轉殖豬之研究發展	臺灣養豬科學研究所	朱瑞民	四年
TW-ARS-34	長期食用含低劑量黃麴毒素飼料對豬隻免疫功能、一般性能、血液和血液化學及組織形態的影響	臺灣養豬科學研究所	龐　飛 吳繼芳	三年

㈤第五期(一九九〇)合作計畫

本會於七十九年二月初提送十項合作研究計畫書至美國農業部複審，結果選擇其中七項計畫進行合作，主要合作研究計畫包括：食品加工製造、家畜禽疾病、植物害蟲防治及人工種子等研究。七項合作研究計畫總經費預算為四〇、四一四、二四八元，其中由美方支援二二、四〇四、二四八元，我方支援一八、〇一〇、〇〇〇元。本期合作計畫項目詳如表五。

表五、中美農業科技第五期(一九九〇)合作計畫項目

計畫編號	計　　畫　　名　　稱	執　行　機　構	主持人	期限
TW-ARS-38	本省家禽流行性感冒之監測	中興大學	謝快樂	三年
TW-ARS-43	黃豆水分含量及油品中微量物質對精練油品質之影響	食研所	朱燕華	四年
TW-AES-42	人工種子技術的發展	生技中心	宋台生	四年
TW-AES-44	生物合理性化合物在害蟲防治之應用	中興大學	侯豐男	四年
TW-AES-39	牛邊蟲病流行病學、血清與防治之研究	省家畜衛生所 台糖畜研所	蘇杰夫 徐興鎔	五年
TW-ARS-30	擠壓加工對米製品物理及化學性質影響之研究	臺灣大學	蔣丙煌	三年
TW-AES-40	以脫皮全脂黃豆粉全部利用製造黃豆食品	食研所	陳文亮	三年

(六)第六期(一九九一)合作計畫

本會於七十七年提送林試所研提之香菇栽培計畫，美國農業部於八十年同意列入合作項目，另亦核定中興大學研提之食品檢測技術計畫，執行期限三—五年，兩項計畫列為第六期合作計畫。計畫總經費預算為九、七四八、九〇〇元，其中由美方支援五、二七九、九〇〇元，我方支援四、四六九、〇〇〇元。本期合作計畫項目詳如表六。

表六、中美農業科技第六期(一九九一)合作計畫項目

計畫編號	計　　畫　　名　　稱	執　行　機　構	主持人	期限
TW-FSIS-4DNA	雜交技術之改進及聚合鏈反應應用於食品中沙門氏菌與李斯特菌之檢測	中興大學	曾浩洋	五年
TW-AES-45	利用美國櫟樹小徑木栽培香菇之研究	林試所	鍾旭和	三年

(七)第七期(一九九三)合作計畫

本會於八十二年三月初提出十五項合作研究計畫摘要，包括植物病蟲害、作物育種改良、家畜疾病及遺傳、蝦病診斷、森林經營與規劃等。通過十一項合作計畫項目。總經費預算為四九、六八七、〇〇〇元，由於美方經費短絀，僅擬支援國外差旅費，預算四、二三八、〇〇〇元，我方支援四五、四四九、〇〇〇元。本期合

作計畫項目詳如表七。

表七、中美農業科技第七期(一九九三)合作計畫項目

計畫編號	計　　畫　　名　　稱	執　行　機　構	主持人	期限
TW-AES-49	利用導管侷限性*Clavibacter xyli* subsp *cynodontis* 構築植物系統性遺傳特性轉殖系統	台灣大學	林長平	三年
TW-ARS-46	利用遺傳工程誘導對蘭花喜姆比蘭嵌紋病毒產生抗性之研究	農試所鳳山分所	王惠亮	四年
TW-ARS-47	台灣重要作物Closterovirus 病害之診斷與防治技術研究	中興大學	曾德賜	三年
TW-ARS-48	開發蟲生真菌作為防治農業害蟲用微生物殺蟲劑及其應用研究	屏東技術學院	劉顯達	三年
TW-AES-50	利用分子標誌選育多重抗病毒之熱帶番茄品種之方法研究	亞蔬中心	S.K.G. Green	五年
	天國栗高硫蛋白質基因在毛豆及綠豆的表現	亞蔬中心	宋　達	五年
TW-AES-52	牛指狀絲蟲在台灣之流行病學及防疫研究	台灣大學	費昌勇	三年
TW-AES-15	純種及雜交種肉牛遺傳與環境相關之研究	畜試所恒春分所	成游貴	四年
TW-AES-53	台灣養殖蝦類細菌性疾病之快速診斷及防治研究	台灣大學	陳秀男	五年
TW-FS-15	國有人工林永續生態系經營的研究	林試所	楊政川	三年
TW-AES-54	線性規劃及模糊目標規劃在森林經營計畫決策上之應用	台灣大學	楊榮啟	三年

(八)第八期(一九九四)合作計畫

八十三年十一月本會在美國華府與美國農部國際合作發展署詳加討論之後，確定了第八期(一九九四)四項新合作研究項目(詳如表八)。

表八、中美農業科技第八期(一九九四)合作計畫項目

計　畫　名　稱	執　行　機　構	主持人	期限
1.轉移色素合成基因創造新花色之晚香玉及金花石蒜	台灣省農業試驗所	王強生	五年
	台灣省種苗改良繁殖場	陳駿季	
	國立中興大學	宋濟民	
2.台灣薊馬傳播之蕃茄斑萎病毒群之研究	台灣省農業試驗所	張清安	五年
	台中區農業改良場	陳慶忠	
	國立中興大學	葉錫東	
		邱人璋	
	國立屏東技術學院	陳滄海	
3.構築以豬假性狂犬病病毒為載體之多價疫苗	國立台灣大學	潘銘正	三年
	中央研究院	呂勝春	
		張清華	
4.開發抗傳染性胰臟壞死病毒核醣轉殖魚類	中央研究院	吳金洌	三年
		徐亞莉	
		張繼堯	
	生技中心	陳鐵雄	

㈨第九期(一九九五)合作計畫

本會於八十五年提出兩項合作研究項目（詳如表九）。

表九、中美農業科技第九期(一九九五)合作計畫項目

計　畫　名　稱	執　行　機　構	主持人	期限
1.研發對環境安全且有效之住宅與商業用木製品抗燃劑	台灣省林業試驗所	鄒哲宗	三年
2.豬血清中脂肪細胞分化抑制蛋白之分離及其在養豬上之應用	台灣養豬科學研究所	劉昌宇	四年

五、中美合作舉辦國際研討會

近年國內蔬菜、花卉作物受蕃茄斑點萎凋性病毒(Tomato Spotted Wilt Virus)之危害日益嚴重，本病毒屬蕃茄斑萎病毒群(Tospoviruses)由薊馬傳播，其寄主廣達五百餘種作物，防治甚為困難，是熱帶、亞熱帶地區最重要的植物病毒之一，也是目前國內多種蔬菜花卉生產上的重要限制因子之一。世界農業先進國家如美國、

加拿大、荷蘭、德國等，鑑於本病毒之危害嚴重性，近年對病毒生物學、生態、流行學、蟲媒、與防治之研究均甚為積極，並已就此病毒群舉辦了二次國際性研討會，以促使研究人員交換研究成果與心得，商討防治對策。有鑑於本病毒對國內蔬菜花卉造成的危害和經濟損失益趨嚴重，且本病毒之蟲媒－薊馬更是園藝作物上的重要害蟲，故近年國內研究人員亦極重視本病毒及所屬病毒群。中美雙方在八十三年十一月之諮商中，雙方不僅同意就本病毒進行合作研究，亦同意由中美合作於八十四年在我國舉辦第三次國際研討會，以本病毒群與園藝作物上之其他重要病毒及蟲媒為研討重點。我國於是在八十四年十一月七～十日假台灣省農業試驗所舉開「花卉與蔬菜作物番茄斑萎病毒及媒介蟲薊馬國際研討會」（International Symposium on Tospoviruses and Thrips of Floral and Vegetable Crops），邀請到二十個國家一百一十五位有關之專家學者與會，其中包括該研究領域內之數位國際知名且權威之專家學者。研討項目包括病毒群新生理小種、細胞學、基因組成、抗性育種、抗性之誘導和相關遺傳工程、薊馬及其傳播、病蟲害之管理等，透過專題報告及壁報方式進行。該項研討會成果豐碩，國立中興大學植病系葉錫東教授並被推薦擔任病毒之命名委員，是一項國際殊榮。研討會之專題講稿於會後彙整編輯，並委託國際園藝學會印行專刊，對國際發行。

六、展望

中美簽訂「農業科學合作計畫綱領」為繼續台灣關係法後，在農業方面雙方推動實質合作邁開一大步，此一綱領明定美國農業部及我方農委會為負責執行單位，其對等之官方合作管道，對推展雙方農業技術實質關係意義深遠，有助於加強我國農業國際合作及促進我國農業技術援外工作的基礎。中美雙方自簽訂「農業科學合作計畫綱領」以來，自七十六年至八十六年已合作進行九期計六十一項合作研究計畫，雙方對合作之進展，均表滿意。本項中美合作計畫綱領已執行十年，為對各項合作研究計畫成果作一階段性之彙整，藉以綜合過去成效及經驗，並策劃未來雙方繼續合作之重點方向，訂於八十六年五月再度假台灣省農業試驗所舉辦「中美農業科技合作研究計畫檢討會議」，邀集雙方負責推動計畫人員及合作研究專家學者共同參與，提出專題報告，並交換心得，俾就所獲結論，提供中美雙方未來加強合作研究，及研訂新合作重點之參考。

Recent Development and Perspective of Agricultural Biotechnology in Taiwan

Te-Yeh Ku

Taiwan is an island which covers an area of 13,814 square miles with a high density population of 21 million and poor natural resources.In the past 15 years, its economy has moved rapidly from an initial agricultural base into an industrial society. Recently, under the trends of industrialization, free trade, and international development, Taiwan's agriculture has faced increasing pressure from international competition and production efficiency. The Council of Agriculture (COA) realized that biotechnology would play an important role in promoting agricultural productivity and development, and for this the COA initiated a national research program starting in 1987 to support and assist in many biotechnological research projects which have been conducted extensively in previous years in various research institutions and university laboratories. Due to limited manpower and resources, this program has research projects prioritized, screened and evaluated to ensure the quality and integration of the research project. A brief review in trends of research and development of agricultural biotechnology in Taiwan is presented in the followings.

I. Direction

The following research topics have been targeted in the COA's Agricultural Biotechnology Program:

1. Diagnosis and control of plant diseases and pests

Damages from plant diseases and pests are major limiting factors for crop production in Taiwan. Accurate diagnosis of crop diseases and pests not only helps control but can also prevent their spread. Serological kits and DNA probes are the mainfocus for the development of detection and diagnostic technology. Isolation and transfer of disease resistant genes and development of recombinant viruses against pests are emphasized for disease and pest control.

2. Plant gene transfer technology

This research project is a very important area in agricultural biotechnology. Once an

useful gene is cloned, it can be transferred into crops to make genetic improvements. This project particularly emphasizes genes related to disease-resistance, insect -resistance, flower color, cold-resistance, protein content and high photosynthesis rate.

3. Propagation and improvement of agronomic and horticultural crops

The principles of cell totipotency express that plant tissue, somatic cells or sex cells are capable of regeneration through organogenesis or embryogenesis and transformation into morphological bipolarity. The development of *in vitro* manipulation of morphological transformation has made a great contribution in plant science and agricultural industry. Additionally, the *in vitro* culture technique is also a prerequisite for the development of genetic transformation through plant cells or calli.

4. Propagation of forest crops and production of secondary metabolites

Four indigenous forest crops in Taiwan, viz. *Cinnamomum kanehirae, Taxus mairei, Chamaecyparis* spp., and *Picea morrisonicola,* are studied in this project. The research focuses on using tissue cultures to propagate and rejuvenate superior aged trees for *C. kanehirae* and investigate the taxane contents in the needles of individual tree and to produce taxol for *T. mairei.* In addition, for *Chamaecyparis* spp. this project tends to reproduce artificial plantlets from in vitro shoots or somatic embryos and to establish an in vitro system via somatic embryogenesis for *P. morrisonicola.*

5. Food biotechnology

The main goal of food biotechnology research and development is to increase the competitiveness of food industries by studying the cloning and expression of food related enzymes and ingredients as well as the improvement of expression of vector-host systems.

6. Aquaculture

The application of biotechnology in aquaculture has been categorized into four topics according to human resources as well as research interests in Taiwan, which cover: (a) Application of biotechnology to enhance fish adapatation to the environment; (b) The development of transgenic aquacultural animals; (c) Cryopreservation of embryo of finfish and shellfish; (d) The development of diagnostic kits for shrimp diseases and development and evaluation of vaccines for shrimp and fish diseases.

7. Animal and veterinary science

Biotechnology offers tremendous potential for improving animal production. Research areas of higher priorities for the sake of solving present problems and effectively using resources include: (a) the development of diagnostic kits for animal diseases and for food safety inspection; (b) recombinant vaccines for the prevention of economically important animal diseases; (c) embryo transfer and genetic modification of domestic animals; and (d) engineering of microorganisms for reducing the pollution caused by animal waste.

II. Current Status and Major Achievements

1. Diagnosis and control of plant diseases and pests

The problems of crop damages caused by plant diseases and pests have been increasing in recent years in Taiwan. In order to diagnose the causes of crop damages accurately and quickly so that remedial measures may be taken promptly, biotechnology is employed to develop monoclonal antibodies and DNA probes for the detection and identification of plant disease causal agents. It is also used to develop measures for controlling diseases and pests. Presently, several monoclonal antibodies were obtained to detect viruses such as bamboo mosaic virus, banana bunchy top virus, and to detect phytoplasmas of peanut witches* broom and sweetpotato witches* broom. The ELISA techniques were applied to field survey and detection, establishment of healthy seed/seedling programs and detection of mild strain viruses for cross protection. In addition, DNA probes were also acquired for plant pathogen detection as well as to increase sensitivity of disease diagnosis. Identification of smut fungi and detection of *Pythium* spp. in hydroponic culture are two examples.

2. Plant gene transfer technology

There are 4 subtopics under this title:

(A) Transformation of disease-resistant genes in horticultural and agronomic crops

Agrobacterium-mediated transformation system of tomato, pepper, sweet melon, water melon, cucumber, and papaya has been established primarily using GUS reporter and disease-resistant genes. Three transgenic lines of papaya containing PRSV coat proteins (CP) were screened and showed different levels of resistance to the virus. Peppers transformed with a truncated cucumber mosaic virus (CMV) showed either no signs of CMV infection or showed milder symptoms than untransformed plants. In addition, Bt insecticidal crystal protein genes were transferred into cauliflower, cabbage, broccoli and Chinese cabbage via *Agrobacterium* spp.

(B) Quality improvement of *Phalaenopsis* cut flower utilizing biotechnology

An ACC synthase gene from *Phalaenopsis* has been isolated and sequenced. Plasmids for gene transfer containing the sense and antisense ACC synthase sequence have been constructed as well.

(C) Construction of linkage map based on RAPD and RFLP markers in sweet melon

Constructing a genetic linkage map for sweet melon is in progress using RAPD, RFLP, and isozyme markers. Two hundred and twelve mapped cabbage, tomato and tobacco clones against sweet melon genomic DNA have been probed to establish STSs.

(D) Resistance breeding of transgenic banana against bunchy top virus (BBTV)

One clone of replicase gene from banana bunchy top virus was sequenced and constructed into a plant gene expression vector in antisense orientation.

3. Propagation and improvement of crops

(A) Massive production of quality nurseries

The *in vitro* technology of plant tissue culture has diverse advantages stimulating the progress in plant propagation and improvement. It has become routine technology in the massive production of quality nurseries. It is particularly useful in vegetatively propagated crops such as banana, papaya, passion fruit, citrus, potato, strawberry, orchids. It is worth mentioning that the micropropagation of banana is already extended to most producing countries. Two million banana plantlets can be produced in one crop season in Taiwan and 16 million total each year, which is so far the best record in the world.

(B) Production of artificial seeds or F_1 hybrid clones

Recently, the factors in the determination of embryogenic cells are much understandable and manipulative. The suspension of embryogenic cells destining to somatic embryogenesis could be homogeneously induced in banana and papaya, obtaining a high rate of somatic embryo plantlet conversion. However, the induction of physiological dormancy and encapsulating materials are critical to the development of artificial seeds. It is expected that the development of a high efficiency cell and somatic embryo culture system would be potential in production of artificial seeds or F1hybrid clones. DNA fingerprinting has been proven a powerful tool in assaying somaclonal variation.

(C) Establishment of in vitro conservation system

The *in vitro* culture is also useful in rescuing rare and endangered species. The *in vitro* culture also performs as an efficient technique for genetic resource conservation. It is particularly important for the establishment of a clonal germplasm bank in vegetatively propagated crops. Hundreds of species are preserved in minimum growth storage.

(D) Application of *in vitro* breeding

The applications of *in vitro* breeding in either sexual or asexual crops have made some progress. The combinations of somaclonal variation, in vitro mutagenesis, and selection pressures greatly increase opportunity for obtaining desirable mutants, suchas disease resistance achieved in strawberry and *Cavendish* banana. The anther culture of *Brassica* has regenerated haploid plants. The *in vitro* technique is also very useful in overcoming sexual barriers such as incompatibility.

4. Food biotechnology

(A) Development of the novel amylolytic yeast used in food industry

Due to its rapid growth rate and high ethanol tolerance, brewing yeast *Saccharamyces cerevisiae* has been widely used in alcohol, wine and soy sauce manufacturing. However, the absence of starch digestion ability greatly limits its application. A gene encoding the isoamylase was cloned from *Pseudomonas* spp. by PCR.

To further hydrolyze linear starch (amylose) to glucose to facilitate yeast fermentation, a glucoamylase gene was also cloned from *Aspergillus* spp. This gene was then site-specifically integrated into the *S. cerevisiae* chromosome by integration plasmid with the help of homologous ribosomal DNA. This specially designed yeast showed great potential in light beer and soybean sauce manufacturing.

(B) Production of natural pigment by plant bioreactor culture system

There is a large demand for natural red and yellow pigments on the domestic market. We therefore intend to develop plant bioreactor culture systems to lower the cost.Sterile callus of red beet and purple carrot were established. Optimal medium composition and bioreactor operating conditions were investigated to promote the pigment synthesis of purple carrots and red beets.

(C) Enhancing the utilization of food waste by genetic engineering methods

Cellulose is by far the most abundant carbohydrate available from plant biomass. *In vitro* reconstitution of cellulosome from two recombinant components is under study. The accomplishments of this work shall greatly benefit cellulose degradation research and applications.

Utilization of chitin, another important food processing waste, is also under study. A strong chitin degradation bacterium was isolated from a local sea food identified as *Aeromonas hydrophila.* A 4.5kb corresponding chitinase gene was cloned and expressed in Escherichia coli. Not only can chitinase be used in food waste recycling, but also it has many potential applications in pharmaceutical ingredients and functional foods production.

5. Propagation of forest trees and production of secondary metabolites

Cinnamomum which is an indigenous forest tree. For *C. kanehirae* clonal propagation and somatic embryogenesis have been successfully established *in vitro* by using plants derived from rootsprouts and seeds. For *T. mairei* selection of superior trees containing high taxanes in their needles, embryo-derived plants *in vitro,* and the cultures of callus and cell suspension have been achieved.

6. Aquaculture

(A) Application of biotechnology to enhance the adaptation of fish to the environment

When the cold front passes each year, a large number of cultured fishes die, resulting in losses of hundreds of millions of NT dollars. This project proposes to use biotechnology to study the tolerance of fish under cold stress and to learn the mechanisms for avoiding losses in the future.

The cold tolerance of milkfish was enhanced, and the possibility of the addition of highly unsaturated fatty acid and antifreeze protein as feed additive to improve the cold tolerance of milkfish was evaluated.

(B) Development of the transgenic finfish and shellfish

For gene transfer techniques, a feasibility study on transferring growth hormone genes into finfish, scallop and shellfish has been conducted.

(C) Cryopreservation of the embryos of finfish and shellfish

Studying factors affecting the cryopreservation of the embryos of finfish, shrimp and shellfish may present the opportunity to understand and solve the problem. The tol-

erance of the embryos and the fertilized eggs to antifreezers and its osmotic adjustment is a subject of interest.

(D) Development and evaluation of the diagnostic kits for shrimp diseases

MBV and WSBV are common viruses causing diseases in the artificial propagation of young shrimp. It is therefore an urgent need to investigate the early infection of MBV or WSBV in order to control or treat these diseases.

(E) Development and evaluation of the vaccines for shrimp and fish diseases

The vaccine for eel is made from a mixture of dual bacterial pathogens. This project is aimed at understanding the relationship among the bacteria dosage, frequency of treatments and antibody titer of the oral or immersed vaccines. The major bacterial pathogens of shrimp were also developed into a vaccine to combat shrimp diseases in the hope of reducing the death rate during the artificial propagation.

7. Animal and veterinary science

(A) Diagnostic methods and the development of related diagnostics

The monoclonal antibodies against economically important diseases such as Newcastledisease, pseudorabies, hog cholera, and actinobacillus pleuropneumonia, were produced and/or collected for developing the diagnostic kits. The ELIZA diagnostic kit for detecting the residue of sulfamethazine, antibiotics for pork, was also developed.

(B) Vaccine development by genetic engineering

Research has continued to develop recombinant vaccines such as pseudorabies genedeleted vaccine and live vector vaccines for pseudorabies.

(C) Animal breeding

Various biotechnologies including artificial insemination, super-ovulation, estrous synchronization, embryo transplantation, in vitro maturation and fertilization, embryo pitting and sex determination of split embryos, nuclear transplant, embryo cloning, and injection of genes into newly fertilized eggs have been well established and applied for years. Some real benefits have also been gained, especially with the embryo transfer of cattle.

III. Perspectives

1. Diagnosis and control of plant diseases and pests

Researches will be continued to produce monoclonal antibodies and DNA probes for early detection of plant pathogens and diagnosis of plant diseases with accuracy and promptness. Development of diagnostic techniques will also be conducted to prevent the spread of plant disease and to assist in the production of pathogen-free healthy seeds/seedlings. To develop plant disease and pest control measures, researches will be devoted and expanded to identify resistant genes to produce resistant transgenic plant varieties and to develop microbial control agents.

2. Plant gene transfer technology

Despite strong research programs, commercial utilization of research results are necessary for the development of the biotech industry. Creation of more productive varieties of agriculture and horticulture crops which could be utilized on a commercial scale is the ultimate achievement in the development of biotech.

Likewise, the transformation of research techniques, useful products or newly developed cultivars from research institutes to farmers or producers is also important. All the products from upstream research must be market-oriented and will have to be applicable in a less sophisticated scientific environment, as well as in mass production in order to be transferable as soon as possible to the applied level.

3. Propagation and improvement of horticultural crops

We still have some limited knowledge regarding genetic control of yield or quality. However the highlight of in vitro manipulation in cell genetic transformations has become feasible. The regenerative capability of plant cells should be inducible in the most major crops, not just focus on the model plants. The efficient cell and somatic embryo conversion system would be of benefit for engineering cells. The deliveries of foreign genes into embryogenic cells through various vectors have progressed in banana and Brassica. The regenerants were assessed to be transgenic plants.

4. Food biotechnology

In addition to the gene recombination and tissue culture research mentioned above, industrialization of microbial production of food product quality will also be emphasized in the food biotechnology sector in the future.

5. Propagation of forest crops and production of secondary metabolites

For *C. kanehirae*, rejuvenation of old superior trees for clonal propagation is economi-

cally important. For *T. mairei,* both selection of individual trees with needles containing high taxol and 10-deacety baccatin III and production of taxol from selected cell lines are economically feasible. For *Chamaecyparis* spp., clonal reproduction using artificial seeds is a time- and money-saving method in comparison to the traditional method via cutting. For *P. morrisonicola,* establishing a system of somatic embryogenesis is a useful technique for large scale propagation.

6. Aquaculture

The value of aquacultrue in our country has comprised a large share of total fishery output. However, due to an increasing pollution problem, epidemics of shrimp diseases, and so forth, have caused severe difficulties for further development of aquacultrue in Taiwan. Fortunately the rapid development of biotechnology may be applicable in aquaculture to provide space for development, for example: breeding improvement, improving the cultural environment, disease diagnosis, and the development of vaccines of high potency.

7. Animal and veterinary science

The following research and development areas have higher priorities for the sake of solving present animal industry problems and effectively using resources.

(a)Development of diagnostic kits for animal diseases.

(b)Development of diagnostic kits for food safety inspection.

(c)Development of animal vaccines and their related adjuvant and assay technologies for the retention of economically important animal diseases.

(d)Embryo transfer and genetic modification of food producing animals.

(e)Engineering of microbes for reducing the pollution caused by animal waste.

8. Biopesticides and biofertilizers

In addition to those mentioned above, the Council of Agriculture has just initiated a research program to develop biopesticides and biofertilizers.

Biopesticides as natural pest control agents are valuable because they are environmen -tally friendly and target-specific. They can be used to partially replace toxic chemical pesticides presently applied extensively in the field. For the time being, development of bioinsecticides and biofungicide is targeted.

Biofertilizers are beneficial microorganisms which can facilitate the uptake of nutrients by crops, and help cutting down the need for chemical fertilizers. The major emphasis is to improve the capability of some potentially useful biofertilizers, such as nitrogen-fixing microbes, mycorrhizal fungi, phosphorous-solubilizing microbes, and

thermophilic cellulose-decomposing microbes. The ultimate goal of the researches is to improve microbes to have composite functional mechanisms in facilitating nutrient uptake.

In order to promote the application of biotechnology, a plan called "Bio-technology Upgrading Program" was issued by the Executive Yuan, ROC. In the area of agriculture, the plan has prioritized flower, animal vaccine, and biopesticide for development. It is expected that related biotechnology industry will be established in a 3-5 year time table. To implement the plan, the Council of Agriculture (COA) has set up three working groups, one for each category, and held meetings with people from research and industry sectors to discuss on how to meet the needs of and to provide technical assistance to the industry for commercial manufacture of biotech products. The followings describe current development of the three categories:

I. Flower Industry

In Taiwan, the application of biotechnology on modern flower industry is mainly focused on the micro-propagation for mass production of healthy young plants or seedlings by the use of tissue culture technique. To use the technique of gene transformation for creating new varieties is still on the stage of research, and mainly performed by government supported institutes. In micro-propagation, owing to the general know-how is easy to acquire and the facilities are easy to install, quite a few nurserymen have been engaged in this field. Today there are more than 200 producers in total, however, only less than ten are performing large-scale production. Most producers are running their business on a small-scale level, and are of part time or family companies. Among the nursery business in all flower crops, Phalaenopsis, with self-developed varieties and leading technique, has become the most important kind. There are millions of seedlings in Phalaenopsis produced for export every year. The next in order are cymbidium, gerbera, anthurium, oncidium, cypripedium, and some foliage plants. Recently, several kinds of bulb flowers (bulbs or tubers) such as lily, calla, gladiolus, Lycoris, and amaryllis have also been put a high premium on R&D. A Technical Service Team, composed by the expert in different fields including biotechnology, floriculture, agricultural engineering, plant pathology, and postharvest, has been organized by COA since 1996. Its goal is to help nurserymen to overcome their technique bottleneck and to lead investors to a better business management. The service is focused on the orchids and bulb flowers, in order to produce high quality and high price seedlings. To help managing tissue culture product, a computer operation system has been successfully set up by the

service team, and its technique has been passed over to the related producer.

Right now the emphasized items for industrial development are as following:

1. The breeding and extension of the newly developed flower crops, in order to create self-developed varieties which are very crucial for the prosperity in the flower industry.
2. The establishment of automation system in bulb flowers propagation, in order to produce healthy bulbs in large scale and reduce the production cost.
3. The qualification and registration for orchid variety and seedlings, and the improvement of mass propagation technique for the development of orchid nursery industry.
4. To assist and guide flower nursery companies to enhance R&D and to breed new varieties, in order to produce healthy and true-to-the-type seedlings, and to establish a well organized flower industry for international marketing.

II. Vaccine Industry for Animal use

Animal vaccines play very important roles on animal disease control and the development of animal industry. It is a technique intensive industry, and every country in the world is paying more and more efforts for its development.

Most of the animal vaccines used in Taiwan are imported from foreign countries. The market share of local made vaccines is only about 25%. In Taiwan, there are 8 animal vaccine manufactures, and all of their production scales are so small. Since all of them are lacking of abilities for the development of vaccines, and their research resources are only from universities or research institutions, the vaccines they can produce are from traditional technologies. Due to the insufficient abilities for the development of new products and the improvement of quality, the difference between Taiwan's and imported animal vaccine value and quality becomes greater and more greater. Consequently, the market share of the local made vaccines decreases very rapidly.

In recent years, animal vaccines production has become a great potential technology, and the market share of vaccines in the Asia Region has also become potent. How to encourage and assist our animal vaccine industry on the improvement of research abilitie s and quality levels, especially on the production of the vaccines for specific diseases which are not endemic in other countries and have great market share potential ones, is an emergent task for us to reduce the influence of being a WTO member countries. The key items fot the development of this industry are:

1. Vaccines produced by the traditional technologies:

   These products include inactive vaccines for controlling Pleuropneumonia as well as Actinomyces and specific disease infections; Bordetella, Pastrella, Actinomyces, Salmonella and Pseudorabies multivalent inactive vaccine; swine Parvo virus inactive vaccine; Hog cholera and Erysypella bivalent live vaccine; Hog cholera and Japanese encephalomylities bivalent live vaccine; Porcine Reproductive and Respiratory Syndrome live vaccine; Infectious Brochities live vaccine; Newcastle disease, Infectious Bursal disease, Egg drop syndrome, Infectious bronchities, coryza and Escherichia coli multivalent inactive vaccine.

2. Vaccines produced by new biotechnologies:

   These products include gene deletion pseudorabies live and inactive vaccines.

3. The improvement on the preparation of adjuvant:

   Adjuvant is the most important ingredient for animal vaccines. Since they are strongly related to the quality and the efficacy of vaccines, more attentions should be paid to their development.

III. Biopesticide Industry

Damages caused by diseases and insect pests is one of the limiting factors in agricultural production in Taiwan. Due to the rapid increase of importation of agricultural products and development of horticultural industry, the incidence of new diseases and insect pests have occurred. To reduce the damages, pesticide application has been adopted as major control method. This has resulted in great concern about pesticide residue and environmental pollution. The current policy in plant protection is to develop accurate diagnostic technology for proper control of diseases and insect pests, and to explore non-pesticidal control measures. Developing biopesticides to partly replace the uses of pesticides is a worldwide trend in plant protection. Biotechnology has provided a powerful tool for that purpose. In order to protect crops, The COA has granted researches to develop antibodies and nucleic acid probes for fast and accurate detection of disease causal agents, and to employ genetic engineering technology to develop gene transferred plants with resistance or vaccines for cross protection.

Presently the aims of biopesticide development are to improve methods for screening organisms with high protection efficiency, for instance, nematodes and microorganisms including viruses, fungi, and bacteria, as well as to develop technology and formula for mass propagation and fermentative production in order for further establishment of processes for

commercial manufacture. This will facilitate the efficient use of biopesticides in Taiwan. The major points of current development as follows:

1. Collecting and improving local *Bacillus thuringiensis* (Bt), establishing technology for mass propogation, and developing Bt insecticide.

2. Improving methods to screen insect-viruses, entomopathogenic fungi, nematodes and developing fermentative production models for bioinsecticide development. The target organisms include nuclear polyhodrosis virus, *Steinernema* spp., *Beauveria bassiana, Metarhizium anisopliae,* and *Nomuraeu rileyi,* etc.

3. Screening elite virus strains and antigonistic microorganisms from soils or leaves, for example, *Trichoderma* spp. and fluorescent pseudomonads, establishing fermentative production models and formula, and developing biofungicides for disease control.

中美農業科學合作研究計畫執行結果報告

Final and Progress Reports of COA-USDA Agricultural Science Cooperative Projects

農業領域 (Agriculture)

一、計畫名稱：

中文：大豆油耐高溫之研究

英文：Improvement of soybean oil at high temperature

二、執行期限：1987年7月1日至1991年6月30日

三、執行單位：食品工業發展研究所

四、美方合作單位及人員：

Food Quality and Safety Research, NCAUR, ARS, USDA

Mr. Timothy L. Mounts

五、中方研究人員：朱燕華

六、計畫目的：

1. 探討各種添加劑包括抗氧化劑、螯合劑和矽油以及氫化製程中各操作條件對大豆油安定性之影響。
2. 探討大豆油在君熱及炸製程中的理化性質之變化以建立正確及快速的方法作為評估大豆油油炸時品質之指標。
3. 評估工業界提昇油脂安定性量產製程之可行性。

七、執行成果摘要：

1. 單一及調合式大豆油在加及油炸時品質之限值可由介電常數、總極性物質、折射率、油炸係數、酸價、碘價、茴香價、羰基價、粘度和顏色等測定方法判別之。
2. 若干商業用鎳觸媒的活性可由活化能的測定判別之。Resan 22、G95E、Nysosel 222和325具低活化能且低亞油酸選擇性；DM3具高活化能且高亞油酸選擇性。
3. 大豆油中的微量物質諸如含磷化合物、氧化物、胡蘿蔔素和鐵金屬對於氫化製程中所使用的鎳觸媒之活性具有毒化作用。

八、英文摘要：

The stability of soybean oil was improved by addition of additives, blending with more stable oils, and hydrogenation. The methods for evaluation of oil deterioration include changes in dielectric constant, total polar compounds, refractive index, fritest, acid value, iodine value, anisidine value, carbonyl value, viscosity and color. When Food Oil Sensor reading of 4 as a measure of changes in DC of oil has been suggested as the criterion of low oil quality, the cut-off level for straight and blended soybean oil were thereby identified by using the above parameter.

During hydrogenation of soybean oil, temperature has great effect on the reaction rate, trans isomer and selectivity ratio. The activities of several commercial nickel catalysts were determined by measuring their activation energy. Among these catalysts, G95E, Resan 22, Nysosel 222 and 325 with low activation energy were more active than

DM3 and G95H which had higher activation energy. However, the less active catalysts such as DM3 and G95H increased the linoleate selectivity of soybean oil during hydrogenation. The yields of both trans isomers and winterized oil were higher for the more selectively hydrogenated oil catalyzed by the less active catalysts. In the sensory evaluation, the fractionated solid fat which contained more trans isomers was lower in flavor scores than the fractionated liquid oil after hydrogenation and winterization of soybean oil.

The poisoning effect of minor compounds of soybean oil on the activity of nickel based catalysts during hydrogenation was investigated. High amount of phospholipids, oxidation products, □-carotene and iron present in the oil reduced hydrogenation rate. Among the different processes of soybean oil before hydrogenation, the hydrogenation rate of refined and bleached (RB) soybean oil was high when this oil was prepared by water degumming, neutralization and bleaching with activated clay in sequence. As soybean oil prepared by either water or acid degumming combined with neutralization, or bleaching with neutral clay, the hydrogenation rate was reduced due to the high amount of minor compounds present in the RB soybean oil, which were not efficiently removed during refining and bleaching processes.

九、已發表報告目錄：

1. Chu, Yan-Hwa. 1991. A comparative study of analytical methods for evaluation of soybean oil quality. *J. Am. Oil Chem. Soc. 68(6)*:379.
2. Chu, Yan-Hwa and Li-Hui Lin. 1991. An evaluation of commercial nickel catalysts during hydrogenation of soybean oil. *J. Am. Oil Chem. Soc. 68(9)*:680.
3. Chu, Yan-Hwa and Li-Hui Lin. 1992. Effects of minor compounds on hydrogenation rate of soybean oil. *J. Am. Oil Chem. Soc. 69(9)*:880.

一、計畫名稱：

中文：大豆油微量物質對精製油品之影響

英文：Effects of minor constituents of soybean oil from soybeans of various moisture content on the quality of RBD soybean oil

二、執行期限：1990年7月1日至1992年6月30日

三、執行單位：食品工業發展研究所

四、美方合作單位及人員：

Food Quality and Safety Research, NCAUR, ARS, USDA

Mr. Timothy L. Mounts

五、中方研究人員：朱燕華

六、計畫目的：

1. 探討不同品質之大豆其微量物質含量之差異性及在精製過程變化與對成品油品質之影響。
2. 探討如何在精製步驟中提高或降低對成品油具有正負面影響之各微量物質。
3. 對不同品質之大豆尋求最佳之前處理方式以獲得最佳之成品油。

七、執行成果摘要：

1. 大豆油水份含量之多寡對大豆油中生育酚之含量與油色有很大的影響，水份含量較低的大豆所提的油含有較高含量之生育酚，油脂的安定性較好且油色較淺。高水份大豆或壓破的大豆，其大豆品質雖較差，但若在大豆壓破碾片前予以蒸氣處理亦可得到品質良好之精製大豆油。高水份大豆所提之粗油油色較深，但其β-胡蘿蔔素含量卻很低，足見具橙黃色之β-胡蘿蔔素與油色沒有直接的關係。
2. 在粉末狀態下提取大豆粗油，提油率高且粗油中含有較高之生育酚。豆片厚度與提油率有密切關係，以較薄之豆片提油，提油率高但其生育酚含量較低。
3. 原料大豆中破碎豆比例愈高者，粗油酸價愈高，生育酚含量愈低。同時水分含量愈高者，隨貯存時間增長，酸價上昇及生育酚含量下降之情形愈明顯；提油系統中有已氧化油脂存在時，則粗油中生育酚含量明顯降低。

八、英文摘要：

The quality of soybeans has great influence on the minor compounds during oil processing. Soybean oil from highly moisted beans or cracked beans contains high amount of nonhydratable phosphatides. The total tocopherol content was greatly reduced for the oil from highly moisted beans. Damaged soybeans subject to steam pretreatment can improve the oil quality by maintaining high amount of tocopherol but low amount of nonhydratable phosphatides. The high acid values in RBD soybean oils from highly moisted and cracked beans was due to the high content of mono and diglycerides in crude

oils.

The conditions of soybeans prior to extraction affected the tocopherol content of crude soybean oils. Optimum thickness of soybean flakes resulted in lowest residual oil level in the flakes and higher amount of tocopherol retained in the oil. Higher moisture content and longer storage periods of soybeans resulted in lower tocopherol content in crude oils. Soybean flakes contaminated with oxidized oil had significant effect on the reduction of tocopherol content in crude oils. Crude soybean oil containing high amount of phospholipids which acted as antioxidants resulted in a less decrease in the tocopherol content of oil during heating.

九、已發表報告目錄：

1. Chu, Yan-Hwa and Jiuh-Yaw Lin. 1993. Factors affecting the content of tocopherol in soybean oil. *J. Am. Oil Chem. Soc.* 70(12):1263.

一、計畫名稱：

中文：降低果實貯藏寒害之研究

英文：Reduction of chilling injury in fruits

二、執行期限：1987年7月1日 至 1990年6月30日

三、執行單位：國立台灣大學農學院園藝系

四、美方合作單位及人員：

HCQL-ARS-USDA Beltsville, Maryland

Dr. Chien-Yi Wang

五、中方研究人員：王自存

六、計畫目的：

低溫貯藏為延長水果蔬菜採收後貯運壽命最重要而且是最常用之方法，但是本省許多屬於熱帶及亞熱帶之水果，當貯存於10 - 12℃ 以下之低溫時往往會發生由於低溫所引起之寒害，而使其品質劣變，貯運壽命減少。本計劃選擇橫山梨及椪柑二種具有重要經濟價值且在貯運過程中經常需要冷藏處理之水果，研究造成其寒害發生之條件、徵狀及減低寒害之方法。

七、執行成果摘要：

(1)橫山梨果實寒害之徵狀與其發生條件及減低寒害之方法：橫山梨為對低溫極為敏感之果實，當採收後貯藏於0℃中5天，再移至20℃ 3天後，便有50%之果實出現寒害之徵狀。橫山梨之寒害徵狀全部發生在果實內部，起初為果肉出現透明或褐色之水浸狀小區域，隨低溫之時間愈久，果肉褐變之面積愈大，終至整個果實均褐化。在0℃下13日後所有的果實均發生寒害，其褐化面積約為全果之80%。寒害發生之溫度以0℃最為迅速與嚴重，5℃次之，即使在10℃下，經20天後亦有約20%之果實出現輕微之徵狀。依採收季節而分，正期梨〔8月初月初〕發生寒害之情形最為嚴重，在0℃中9天即達100%; 4月採收之冬梨在0℃中10天後僅10% 出現徵狀，移溫3天後有60%果實出現寒害; 6月採收之倒頭梨則較冬梨略為敏感，在0℃ 10天有50%出現褐化，移溫後增加到60%。正期梨中採收期愈晚者發生寒害之情形愈嚴重。 不同寒害褐化程度之果實其徵狀愈嚴重者，電解質滲漏〔Electrolyte leakage〕程度愈大。減輕寒害發生之方法以溫度管理為主，橫山梨之最適貯藏溫度為12.5℃，在10℃中以塑膠袋配合乙烯及二氧化碳吸收劑，可以有20天左右之貯藏壽命。

(2)椪柑果實寒害之徵狀與其發生條件及減輕寒害之方法：椪柑為本省秋冬季之主要水果，當其在10℃以下之溫度冷藏時，通常會發生寒害。其寒害之徵狀主要發生於表皮，起初為局部之油胞周圍組織褐化並出現表皮凹陷之小斑點，嚴重時小斑點擴大為燙斑(scald)，甚至果皮水腐(watery soft)。此寒害徵狀在移至20℃後都會轉趨嚴重。中，低成熟度之椪柑對低溫之敏感程度較高熟度者為高，低成熟度(九成綠)及中成熟度(五成綠)之果實在0℃中貯放25天後移至20℃ 10天後即有凹點及燙斑出現，高成熟

度之果實要到40天之後才會出現輕微的凹點。在0℃中果實之轉色甚差，但在移至20℃後略可改善。椪柑放在PE袋中摹擬MA貯藏，雖然可延遲轉色，但卻不能減輕寒害徵狀之出現；而且在0℃中貯藏到76天後，大部分果實的果皮都呈現水腐，移到20℃後，很快就發霉腐爛。以50 ppm ABA處理，並沒有減輕寒害徵狀，也沒有延遲果皮轉色之時間。用10 ppm乙烯處理，則會促進寒害徵狀發生並加速果皮轉色。

八、英文摘要：

(1)Occurrence, symptom development and reduction of chilling injury in 'Heng-Shan' Pear: 'Heng-Shan' pear (*Pyrus pyrifolia* Nakai var. 'Heng-Shan') is very sensitive to chilling temperature. When mature fruit were stored at 0℃ for 5 days and moved to 20℃ for 3 days, 50% of the fruit developed chilling injury symptom. The chilling injury symptom of 'Heng-Shan' pear occurred in the flesh and core of the fruit. Initially, small patches of translucent and browning areas appeared in the flesh; upon extending the duration of exposure to low temperature, severe flesh browning developed until the entire internal part of the fruit is watery brown. Fruits that stored at 0℃ for 13 days all developed chilling symptom, with more that 80% of the cut surface were brown. Chilling injury symptom occurred most rapidly and severely at 0℃, and less severe at 5℃; even at 10℃, 20% of the fruit showed slight chilling injury symptom after stored for 20 days. The occurrence of chilling injury in 'Heng- Shan' pear harvested at various seasons and/or at different maturities were compared. Chilling injury symptom appeared most severe in normal season pears. Fruits that were harvested in late September developed chilling injury when stored at 0℃ for 5 days and 100% of the fruits showed browning symptoms after stored at 0℃ for 9 days then moved into 20℃ for 3 days. The winter pears were least sensitive, only 10% fruits showed chilling symptoms when stored at 0℃ for 10 days, and increased to 60% after moved into 20℃ for 3 days. The reversed season pears were slightly more sensitive than winter pears; 50% fruits showed chilling symptoms when stored at 0℃ for 10 days and increased to 60% after moved into 20℃ for 3 days. In normal seanson pears, the later the harvesting date, the more severe the chilling injury symptom. It was found that fruits with higher degree of flesh browning had higher percentage of electrolyte leakage. Temperature management is suggested as the major way of reducing chilling injury in 'Heng-Shan' pear. Its optimal storage temperature is 12.5℃; fruits that were stored at 10℃ in PE bags in the presence of ethylene and carbon dioxide absorbent had 20 days of storage life.

(2)Occurrence, symptom development and reduction of chilling injury in 'Ponkan' tangerine : 'Ponkan' (*Citrus reticulata* Blanco var. 'Ponkan') is an important citrus fruit during fall

and winter in Taiwan. The fruit developed chilling injury symptom after stored at temperature below 10℃ for a period of time. The chilling injury symptom occurred mainly on the surface of the fruit. Initially, part of the tissues that surrounding the oil cells became brown, and small sunken pitting area appeared on the surface. Usually, these symptoms were not apparent when examined immediately after cold storage, but developed rapidly into visible pittings and scalds after several days in room temperature. Sumptoms occurred mainly in fruit stored at 0℃, with fruit at 5℃ less severe. Low and medium maturity fruit developed pitting and scald symptom after stored at 0℃ for 25 days then transferred into 20℃ for 10 days. High maturity fruit developed slight pitting after stored at 0℃ for 40 days. These results showed that low maturity fruit were more sensitive to chilling temperature than higher maturity fruits. In addition, chilling temperature slowed down or prevented green fruit from turning yellow; however, moving fruits into 20℃ improved the color change. Storing 'Ponkan' in PE bags to simulate modified atmosphere storage retarded color development but had no effect on reducing chilling symptoms. Most fruit developed watery rot after stored at 0℃ for 76 days and decayed rapidly after moved into 20℃. Treating fruit with 50ppm ABA did not reduce its chilling injury, nor had any effect on color development. Treating fruit with 10ppm ethylene will aggravate its chilling injury, and promote its color change.

圖一、橫山梨果實之寒害徵狀－果肉褐變。

Fig. 1. Chilling injury symptom of 'Heng-Shan' Pear.

圖二、椪柑果實之寒害徵狀－燙斑。

Fig. 2. Chilling injury symptom (Scald) of 'Ponkan'

一、計畫名稱：

中文：烹調與加工對肉品中殘留農藥及藥物之影響

英文：Influence of cooking/processing conditions on pesticide/drug residues in meat and poultry

二、執行期限：77年7月1日至83年2月28日

三、執行單位：國立台灣大學食品科技研究所

四、美方合作單位及人員：

USDA, Science Division, AMS, Washington, DC 20090, U.S.A.

Dr. Robert L. Epstein, Deputy Director

五、中方研究人員：許順堯 教授

六、計畫目的：

本計畫目的在探討烹調與加工對豬肉、雞肉和雞蛋中殘留農藥及藥物之影響。

七、執行成果摘要：

本計畫探討烹調與加工對豬肉中殘留的DDT、飛佈達、陶斯松、氯黴素及左美素，雞肉中殘留的氯四環素，雞蛋中殘留的DDT、飛佈達、陶斯松及氯四環素之影響。結果顯示抗生素藥物如氯黴素比較怕熱，而可以用如製罐之121℃，60分鐘之高熱加以完全破壞。其他農藥及藥物如左美素、DDT、飛佈達及陶斯松則比較耐熱，而無法用一般之烹調與加工方法加以完全破壞。所有之烹調與加工皆多少會降低肉及蛋中農藥及藥物之殘留量。此結果顯示除了加熱裂解外，其他加工因子如：與食品添加物及食物成分產生反應、溶解於水或油中、以及附著於器具上等，皆可能是殘留量降低的原因。

八、英文摘要：

The effects of cooking and processing on DDT、heptachlor、chlorpyrifos、chloramphenicol and levamisole residues in swine muscle tissues, chlortertracycline residue in chicken muscle tissues, DDT、heptachlor、chlorpyrifos and chlortetracycline residues in eggs were investigated. Results indicated that antibiotic drugs, such as chloramphenicol and chlortetracycline were more heat-labile and could be completely degraded by severe heating process, such as heating at 121℃ for 60 min. in canning process. The other drugs and pesticides, such as levamisole、DDT、heptachlor and chlorpyrifos were more heat stable and can not be degraded to its completion by all cooking and processing methods. All cooking and processing methods resulted in decrease of residue level of all drugs to certain degree. Beside heat degradation, the results indicated that many other processing factors, such as reaction with food additives or other food components、leaching into processing water and/or oil and adsorption on processing utensils, could also cause significant reduction on pesticide residue in muscle tissues dur-

ing cooking and processing.

九、已發表報告目錄：

1. Hsu, S. Y. and Epstein, R. L. 1993. Influence of cooking/processing conditions on levamisole rsidues in swine muscle tissues. In "Residues of Veterinary Drugs in Food", N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg(Ed.), 387-390, Netherlands Society for Nutrition and Food Technology, Department of the Science of Food of Animal Origin, Utrecht University, Utrecht, The Netherlands.
2. Hsu, S. Y. and Epstein, R. L. 1994. Processing effects on pesticide residues in swine muscle tissues. Proceedings of the 13th International Pig Veterinary Society Congress. P.363.
3. Hsu, S. Y., Epstein, R. L., Chiou, Huei-jen and Shen, Tian-Fuh. 1995. Depletion of pesticides through chicken eggs. Food Science 22(5):543-549.
4. Hsu, S. Y., Epstein, R. L., Chiou, Huei-jen and Shen, Tian-fuh. 1995. Effects of cooking on pesticide residues in chicken eggs. Food Science 22(5):550-555.
5. Hsu, S. Y., Tang, J. J., Epstein, R. L. and Lee, K. S. 1995. Cooking effects on chloramphenicol residues in swine muscle tissue. Food Science 22(5):528- 532.
6. Hsu, S. Y. and Epstein, R. L. 1996. Influence of processing methods on chlorteracyclin-e residues in chicken muscle tissue. Food Science 23(3):321- 325.
7. Hsu, S. Y. and Epstein, R. L. 1996. Influence of processing methods on chlorteracycline residues in chicken eggs. Food Science 23(4):503-508.

一、計劃名稱：

中文：擠壓加工對米製品物理及化學性質影響之研究

英文：Extrusion cooking of rice — a physical and chemical characteristics study.

二、執行期限：1990 年 7 月 1 日至 1993 年 6 月 30 日，共 3 年

三、執行單位：國立臺灣大學 食品科技研究所

四、美方合作單位及人員：

Southern Regional Research Center, USDA

Mr. James I. Wadsworth

五、中方研究人員：蔣丙煌、郝龍斌

六、計劃目的：

稻米是本省主要的農作物，也是大部份中國人以及亞洲國家人民的主食。但是由於米食消費式樣不多，往往造成供需失調的問題。為推廣本省主要農產品之消費，研究發展米製品是有其必要性的。

擠壓加工為一高溫短時之加工技術，它可將許多食品原料加工為各種形狀、組織、顏色以及風味的產品，為一可用於米製品加工之利器。

在擠壓加工時，原料米處於一高壓、高溫，以及機械性之剪力之下，因此其物理及化學性質方面，均會發生極大的變化。然而，擠壓加工之各種參數對原料米之各種成份究竟造成了何種影響，乃至於這些成份之物理及化學性質的變化與最後產品品質間的關係，均與擠壓米製品之開發息息相關。本研究即針對此一問題，對原料米在擠壓加工中之物理及化學變化及產品品質做一有系統的探討。

七、執行成果摘要：

本研究旨在探討食米在擠壓中成份物質之變化，相互間之作用以及其對產品顏色、香味以及質地的影響。結果顯示，擠壓產品隨擠壓溫度（110 ℃、150℃、190℃）升高，胺基酸及脂肪酸含量皆有下降的趨勢。由於醣類裂解，還原糖在低溫（110℃、150℃）下，含量有上升的趨勢；但在高溫下（190℃），因劇烈褐變使含量下降。因此推測在擠壓過程中，油脂進行氧化；胺基酸與醛酮類（碳水化合物或油脂氧化產物）發生褐變反應，且此二反應皆隨溫度之升高，反應程度加劇。

糙米、白米擠壓產品中的香味成份十分相似。唯白米中香味成份之單位濃度較糙米為高，推測可能是糙米中維生素 E 含量高於白米，造成抗氧化效果。亦有可能是糙米中油脂含量較高，造成在擠壓機中潤滑效果較好，減緩機械效應使然。

當以GC及 GC-MS 分析香味成份，顯示其擠壓產品香味大多來自油脂氧化及褐變反應。油脂氧化產物一般皆得自于油酸及亞麻油酸氧化，有醛類、醇類、醚類、碳氫化合物、酮類、酸類等。褐變產物分析有 pyrazine 類、furan 類等化合物。其中，2-methyl-3-pentylpyrazine 及 2,5-dimethyl- 3-pentylpyrazine，可能來自油脂氧化產物與梅納氧化產物作用而成。

擠壓過程中油脂除了潤滑作用外，尚可參與許多物理性、化學性反應而賦予擠壓產品特有之品質。溫度、螺軸轉速及水份明顯影響油脂之鍵結。在不破壞澱粉結構之前題下，條件愈劇烈，油脂參與的鍵結愈多。在 150 ℃、200rpm、23%下，油脂主要與蛋白質發生交互作用；而 200℃、200rpm、13% 之下，因蛋白質變性及澱粉糊化較劇，使得油脂-蛋白質間之鍵結減少，油脂-澱粉間之鍵結明顯增加。而三酸甘油脂由於其分子較大且缺乏官能基，因此多以微弱鍵結如：凡得瓦力、偶極矩力和食品成份發生作用；而脂肪酸和磷脂質則可和食品成份形成較強之鍵結如：氫鍵、疏水性作用力和靜電吸引力等。整體而言，擠壓過程油脂鍵結對品質之影響並不顯著，但使得擠壓產品有很好之貯藏安定性。

擠壓加工參數，包括溫度、進料水份和螺軸轉速，對物料流變性質以及產品性狀均有影響。實驗結果發現，米在擠壓蒸煮中的流變行為符合power law equation，為一假塑性流體，其中稠度係數隨水份增加而降低，而溫度對其影響則不一定；流體行為係數隨水份的增加、溫度的增加而增加。此外，米在擠壓蒸煮中的視黏度會隨切變速率的增加、溫度的增加與水份的增加而降低，他們之間的關係可以數學模式描述之。模式所算出之視黏度值與擠壓模口壓力有顯著正相關；隨著估計視黏度的增加，擠壓產品水溶性指標有增加的趨勢；估計視黏度與產品程度指標、吸水性指標的關係較不明顯，但固定水份含量時，隨著估計視黏度的增加，產品糊化程度指標、吸水性指標有下降的趨勢，而且水份含量愈高，這趨勢愈明顯；擠壓產品厚度、切斷力隨估計視黏度的增加而增加，但在相同水份含量下，均有隨估計視黏度的增加而下降的趨勢。

八、英文摘要

Extrusion process affects the basic compositions of rice, which, in turn, affects color, flavor, and texture of the extruded rice product. Experinants results showed that the contents of amino acids and fatty acids decreased with increasing extrusion temperatures for both brown rice and milled rice. At lower extrusion temperature (110℃, 150℃), the reducing sugar increased significantly, which might be cuased by polysaccharide hydrolysis. Most likely due to Maillard reaction the reducing sugar decreased at higher extrusion temperature (190℃). The Maillard reaction also resulted in a darker rice extrudate.

Both GC and GC-MS analysis showed that major volatiles generated by extrusion were from lipid oxidation and browning reaction. Both brown rice and milled rice generated similar volatile compounds during extrusion, but different in quantity. More volatile compounds were produced in milled rice. Brown rice, with higher lipid and vitamin E contents had less friction and oxidative reaction during extrusion. Among the volatile compounds, aldehydes, ketones, esters, hydrocarbons and alcohols were generated

from oleic acid and linoleic acid oxidation. Pyrazines and furans were produced from browning reaction of carbohydrate and protein. The formation of 2-methyl-3-pentylpyrazine and 2,5- dimethyl-3-pentylpyrazine were formed by the interaction among products of lipid oxidation and browning reaction.

Lipids not only serve as lubricant during extrusion, but also participate in various physico-chemical reactions, thus affecting the quality of extrudate. The barrel temperature, moisture content of feed, and screw speed were the major factors which affected the binding of lipids with other rice components. In general, more vigorous conditions would result in more interactions. At barrel temperature 150℃, 200 rpm of screw speed, and 23 % of feed moisture content, the rice lipids were primarily interacted with proteins. At barrel temperature 200 ℃, 200 rpm of screw speed, 13%of feed moisture content, possibly due to protein denaturation and starch gelatinization, less interaction was observed between lipid and protein, but the interaction between lipid and starch increased significantly. Because of bulky size and lack of functional groups, the triglyceride formed weak bonding with other components in rice, such as Van Der Waals force and dipole dipersion force. The interactions between free fatty acids and phospholipids, on the other hand, were stronger, their bonding included hydrogen bonding, hydrophobic interaction, and electrostatic interaction. In general, the interactions between lipids and other rice components did not affect the quality of rice extrudate. However, lipid binding increased the stability of rice oil in extrudates during storage.

The process parameters, including temperature, moisture content and screw speed affected the rheological properties and the physical properties of rice extrudate. The rheological behavior of rice in extrusion cooking fitted a power law equation and was pseudoplastic in nature. The consistency index decreased with increasing moisture content. The effect of temperature on the consistency index was less evident. The fluid behavior index increased with increasing moisture content and increasing temperature. The apparent viscosity of rice dough in the die dereased with increasing shear rate, temperature and moisture content. A three parameter rheological model was developed describing their relationship. The estimated apparent viscosity and the die pressure of the extruder had significant correlation. Increasing the estimated apparent viscosity, the WSI of rice extrudate incresed. The effect of apparent viscosity on the extent of starch gelatinization and the WAI was less evident. When the moisture content was the same, the exent of starch gelatinization and the WAI decreased with inereasing apparent viscosity, and this tendency was more obvious while moisture content was higher. The

width and the breaking force of rice extrudate increased with increased apparent viscosity. However they decreased with increased apparent viscosity when the samples had the same moisture content.

一、計畫名稱：

中文：DNA雜交技術之改進及聚合酶鏈反應應用於食品中沙門氏菌與李斯特菌之檢測。

英文：Improving the DNA hybridization technique and application of PCR for rapid detection of *Salmonella* and *Listeria* in foods.

二、執行期限：1986年7月1日 至1991年6月30日，共4年及

1991年7月1日 至1996年6月30日，共5年。

三、執行單位：國立中興大學食品科學系

四、美方合作單位及人員：

FSIS, Microbiology Div. USDA.

Dr. Stanley S. Green

五、中方合作人員：曾浩洋

六、計畫目的：

發展並改進沙門氏菌及李斯特菌的DNA雜交技術及聚合酶鏈反應的快速檢測方法並應用於食品中上述病原菌之檢測。

七、執行成果摘要：

沙門氏菌及李斯特菌係重要的食品病原菌，可造成食品中毒，以標準的微生物檢測方法（BAM方法）檢測上述病原菌，通常費時費力，因此，快速且敏感的檢測方法相當重要。在過去數年，我們發展了食品中沙門氏菌快速檢測用的核酸探針及聚合酶鏈反應。以分子選殖法，我們曾選殖了一段1.8Kb *Hind* III DNA片段，可用以檢測食品，如牛肉、豬肉、雞肉等食品中之沙門氏菌。根據此段1.8Kb DNA片段的DNA序列，我們發展了寡核苷酸片段及聚合酶鏈反應方法，可用以檢測沙門氏菌。同時，我們也設計了呈色式微滴盤式三明治式DNA雜交系統，用以檢測食品中之沙門氏菌。此外，我們也將一些腸內菌的16S rRNA的基因加以定序，經由電腦比對序列方式，設計了以16S rRNA基因為標的的核酸探針及聚合酶鏈反應引子，應用於食品中沙門氏菌檢測。在李斯特菌的檢測方面，我們則使用了文獻報告已有之聚合酶鏈反應引子，此外，我們也發展了沙門氏菌及李斯特菌可同時檢測用的多套式PCR套組。上述DNA探針雜交法之檢測靈敏度為$10^6 \sim 10^7$每一分析，而聚合酶鏈反應方法，檢測靈敏度則為$10^0 \sim 10^1$目標菌每克食品。在檢測時間方面，DNA探針所需時間約需48小時，而聚合酶鏈反應，則僅為一個工作天，比傳統BAM方法所需之5～6天，均要縮短。

八、英文摘要：

Salmonella and *Listeria monocytogenes* are important pathogens which may cause food borne disease. The BAM methods for detection of these bacterial cells are laborious

and time consuming. Thus, rapid and sensitive method for detection of these bacteria is important. In the past years, we have developed DNA probes and polymerase chain reaction (PCR) methods for the specific detection of *Salmonella* in food samples. *A Salmonella* specific 1.8Kb *Hind* III DNA fragment has been cloned and used for the detection of *Salmonella* in food samples, such as beef, pork, chicken etc. Based on the DNA sequence of this 1.8Kb DNA fragment, oligonucleotide probes and PCR primers specific for *Salmonella* detection were also developed. Also, a colorimetric microtiter plate sandwich DNA hybridization system was designed from this 1.8Kb Hind III DNA fragment and used for the specific detection of *Salmonella* in food samples. Furthermore, 16S rRNA genes of several enterobacteriaceae spp. were sequenced and the 16S rRNA gene targeted oligonucleotide probes and PCR primers were designed for the specific detection of *Salmonella* cells. For *Listeria monocytogenes* was detection, reported PCR primers were used. Also, a multiplex PCR system for the simultaneous detection of *Salmonella* and *Listeria monocytogenes* was developed. When detection sensitivities of the above described DNA probes and PCR primers were studied, it was found that $10^6 \sim 10^7$ cells per colony were required for positive DNA hybridization and $10^0 \sim 10^1$ per gram of food sample could be detected when the PCR method was used. Also, the detection time required for DNA hybridization study was less than 48h while the detection time for PCR assay might be shortened to less than one working day. Thus, the detection time would be shortened from 5～6 days (BAM method) to 1～2 days.

九、已發表報目錄：

A : Full papers

(1)Tsen, H. Y., M. H. Chen, J. S. Shieh and N. T. Hu. 1989. The development and application or DNA probe on the detection of *Salmonella.* Proceedings Three Way Faculty Exchange Seminar on the Application of Biotechnology to Agriculture. Kon-Kuk University Press. Seoul, Korea, P.P. 266-273.

(2)Tsen, H. Y., M. H. Chen, J. S. Shieh, S. J. Wang and N. T. Hu. 1989. Possible use of a 1.8Kb DNA fragment for the specific detection of *Salmonella* in foods. J. Ferment. Bioengineering. Vol.68 No. 1.P. 1-6.

(3)Tsen, H. Y., S. T. Wang, B. A. Roe and S. S. Green. 1991. DNA Sequence of a *Salmonella* specfic DNA fragment and the use of oligonucleotide probes for *Salmonella* detection. Appl. Microbiol. Biotechnol.35.No.3 .P. 339-347.

(4)Tsen, H. Y., S. J. Wang and S. S. Green. 1991. Detection of *Salmonella* in meats and fishes by colony hybridization with biotin-labeled DNA probe. J. Food Science. 56:

1519-1523 (USA).

(5)Wang, S. J., M. H. Chen and H. Y. Tsen. 1991. Cloning of two Bam H1 DNA fragments with potential for use in the detection of all Salmonellae, J.Chinese Agric. Chem. Society. 29:No.3. P.258-269.

(6)Tsen, H. Y., Liou, J. W., and Lin, C. K. 1994. Possible use of a polymerase chain reaction method for specific detection of *Salmonella* in foods. J. Ferment. Bioeng. 77: P. 132-143 (Japan).

(7)Tsen, H. Y. 1994. Detection of pathogenic bacteria in foods by using recombinant DNA technique, p. 605-619 in "Recombinant Microbes for Industrial and Agricultural Application" ed by Y. Mrooka. Bioprocss Technol. Vol. 19, Marcer Dekker Inc., N.Y., USA.

(8)Tsen, H. Y., Liou, J. W., and H. H. Hu. 1994. A colorimetric microtiter plate sandwich DNA hybridization system for the detection of *Salmonella* in foods.J.Ferment. Bioeng. 78: p. 12-18 (Japan).

(9)Lin, C. K., and Tsen, H. Y. 1995. Development and evaluation of two novel oligonucleotide probes based on 16S rRNA sequence for the identification of *Salmonella* in foods. J.Appl. Bacteriol. 78: 507-520 (UK).

(10)Liu, P. R., Wang, T. K., Lin, C. K., Pang, T. M., and Tsen, H. Y. 1995. Use of PCR method for the rapid identification of *Salmonella typhi.* J. Food Drug Analysis. 4: 65-73 (Taiwan).

(11)Lin, C. K., and Tsen, H. Y. 1996. Use of two 16S rRNA targeted oligonucleotides as PCR primers for the specific detection of *Salmonella* in foods. J. Appl. Bacteriol. 80: 659-666(UK).

B: Conference papers

(1)Tsen, H. Y. 1989. Possible use of a 1.8Kb DNA fragment for the specific detection of *Salmonella* in foods. International Symposium on Molecular Parasitology, Apr. 24-26, Institute of Biomedical Science, Academic Sinica, Taipei, Taiwan, ROC.

(2)Tsen, H. Y., and S. J. Wang. 1990. Cloning of the DNA probe Specific for the detection of *Salmonella.* The fifth Annual Meeting of Biomedical Science, Paper No.26-150. Apr. 7-8, Taipei, ROC.

(3)Tsen, H. Y., S. J. Wang and S. S. Green. 1991. Sequencing of a *Salmonella* specific DNA fragment and the use of oligonucleotide probes for *Salmonella* detection. The 1991 IFT Annual Meeting, Dallas, Texas. USA. May 29-June 4, 1991.

(4)Tsen, H. Y., S. J. Wang, R. Y. Yang, T. R. Chen and S. S. Green. 1991. XXIV Word Veterinary Congress. World Association of Veterinary Congress: World Association of

Veterinary Food Hygienists Symposium: "Implications of Biotechnolog for World Food Hygiene". Aug.18-23. Rio de Janeiro, Brasil: Chair (Co-chair) and lecturer on "The use of DNA probe and PCR for the specific detection of *Salmonella, S.aureus* and other pathogenic bacteria.

(5)Tsen, H. Y., S. J. Wang, G. K. Lin and J. W. Liou. 1992. Detection of *Salmonella* isolates with all serotypes by polymerase chain reaction. 1992 ASM (American Society for Microbiologists) General Meeting. May 26-30, New Orleans, L.A., USA.

(6)Tsen, H. Y. 1993. "Use of DNA-DNA hybridization and PCR methods for the detection of pathogenic bateria in foods". Invited lecturer. The 4th Japan-POC Symposium on Biotechnology, Tokyo-Osaka, Japan; Jan. 31-Feb. 6, 1993.

(7)Lin, C. K., and H. Y. Tsen. 1993. "Development of a 16S rRNA-based oligomer probe for the detection of *Salmonella* in food samples." 8th Annual meeting for Bio-Medicine, Taipai, Taiwan, ROC. Mar. 20-21. May,1993.

(8)Tsen, H. Y. 1994. March 27-31. Biotechnology on Food Processing. The Third Canada -Taiwan Higher Education Conference. Victoria, British Columbia.

(9)Lin C. K., and Hau-Yang Tsen. 1995. Development and use of 16S rRNA gene targeted polymerase chain reaction for the specific detection of *Salmonella* in foods. The 95th gneral meeting for ASM, Washington, D.C. 21-23. May, 1995.p.44.Session 107.

(10)Lin C. K., and H. Y. Tsen. 1995. Development and evaluation of two novel olionucleotide probes based on 16S rRNA sequence for the identification of *Salmonella* in foods. July 30-Aug. 4, 1995, Budapest, Hungary.

一、計畫名稱：

中文：以脫皮全脂黃豆粉全部利用製造黃豆食品

英文：Total utilization of hulled soybean flour for soybean products

二、執行期限：1990年7月1日 至1992年6月30日

三、執行單位：食品工業發展研究所

四、美方合作單位及人員：

美國農業部北區研究所

Mr. George Bookwalter

五、中方研究人員：黃三龍、侯弘哲、陳文亮

六、計畫目的：開發黃豆新加工方法及拓展黃豆新市場

七、執行成果摘要：

以脫皮全脂黃豆粉代替黃豆製造豆漿和豆腐，好處是可以保留黃豆所有的成份，不必浸豆和過濾，沒有浸豆水和廢棄物，可縮短加工時間。應用反應曲面法以求出最適條件，結果豆漿之最適條件為黃豆粉對水的比例為1比18至24，水溫為55℃至65℃，利用高效率混合機（EBARA Milder MDN-304日製）混合黃豆粉和水，混合後之靜置時間為20至30分鐘，加熱至沸騰。單以黃豆粉為原料製造豆腐時，豆腐之品質很差不被接受。製造豆腐之豆漿必須是黃豆豆漿混合豆粉豆漿，二者的比例為65比35，硫酸鈣的濃度為0.035 M，其他條件與傳統方法一樣。

八、英文摘要：

Hulled soybean flour was instead of soybean to make soy milk and tofu. The benefits are keeping all components in the products, eliminating soaking and filtering, no solid waste, and saving the processing time. Surface Response Methodology was used to find the optimum condition for processing. The results obtained are the soy flour to water ratio is between 18 to 24, the temperature of water is between 55℃ to 65℃, a high efficiency dispersion machine (EBARA Milder MDN-304, Japan) was used to mixing the flour and water, the setting time of the mixture is 20 to 30 min, then cook to boil for soy milk processing. The quality of tofu made from soy flour only is unacceptable. The soy milk for tofu making should be a mixture of soy milk made from soy bean and from soy flour respectively. The ratio of the two soy milk is 65 to 35, the concentration of calcium sulfate is 0.035 M, other conditions are the same as the traditional method.

九、已發表報告目錄：

1. 黃三龍、侯弘哲、陳文亮。1991。以脫皮全脂黃豆粉全部利用製造黃豆食品 (I)，食品工業發展研究所，研究報告第663號。

2. 黃三龍、侯弘哲、陳文亮。1992。以脫皮全脂黃豆粉全部利用製造黃豆食品 (II)，食品工業發展研究所，研究報告第698號。

一、計劃名稱：

中文：臺灣設施栽培系統之研究開發

英文：Engineering protected cultivation systems in Taiwan

二、執行期限：77 年 7月 1日至 82 年 6 月30日

三、執行單位：台大農機系

四、美方合作單位及人員：

Rutergus univerity, Prof. William Roberts,

Dr. Gacomelli, Dr. Ting

三、中方合作人員：吳中興、張漢聖、高德錚、吳文希

六、計畫目的：

(一)引進北美洲最好的加溫型塑料溫室，並將其轉型為亞熱帶型降溫功能之溫室，並兼具抗颱功能。

(二)建立配合該降溫型溫室使用之亞熱帶水耕系統。

(三)建立亞熱帶型溫室之環控系統及微氣候站。

(四)發現並防治該溫室在不同季節可能產生之不同病害。

(五)引進溫室自動化及精緻農業之概念。

七、執行成果摘要：

雙層塑料溫室在北美洲已早享大名，是對植物的生長環境加溫的好設施，然而在熱帶或亞熱帶地區卻從未使用過。為了證實這種溫室同樣的適用於熱帶或亞熱帶地區，從1988年的秋天開始，我們建造了兩座具有相同的尺寸、座向、機械配備及控制系統的雙層塑料溫室，也開始了一個由C.O.A. 及U.S.D.A所支持的長期研究計劃。

我們也建立了一棟氣象站，採用CAMPBELL廠牌的微電腦在氣象站裏收集所需的資料。我們收集的資料包含了室內外各測點的溫度、相對濕度、全幅射能、淨幅射能、光合作用所需要之幅射能及風速、風向等氣候及微氣象資料。

在詳細評估了第一年的實驗數據後，我們發現，溫室內部的實際降溫能力，尚不能符合本省之需要；因此，在第二年的研究計劃裏，我們將上一年度所使用的兩座溫室重新設計並施工。

我們的充氣式雙層塑料溫室，配備有水牆冷卻系統，在結構上並且能夠抵抗強風的猛烈衝擊，試驗的工作，就在各種不同的降溫模式下長年不斷的持續進行著。

在這個全空調的溫室中，裝設動態浮根式水耕系統，來種植葉菜、蕃茄、及胡瓜等作物。水耕系統，作物栽培技術，及幼苗由台中改良場提供。

為了評估減少太陽能幅射能量進入溫室對降溫之影響，在其中一個溫室的屋頂上加裝遮陰網，而另外一個溫室則不裝。

溫室內部的冷卻效果，是由強制對流、蒸發冷卻、遮陰設備以及植物生長的蒸散作用而來。在1990年一個陽光普照的五月天裏，裝了遮陰設備的溫室降溫量是5.5

℃，蒸散作用的冷卻效果與遮陰網相同。兩種設備一起使用時，溫室內部的溫度可由完全不降溫時之55℃以上，降低到30℃以下，這時的溫度，比大氣溫度低。

經由試驗觀察，所有的作物在這個充分空調的系統裏，都長的很茂盛。

在位於彰化縣的台中區農業改良場內，多風的溫室區中，我們蓋了兩座重新更改設計的雙層塑料溫室，並配置了一座氣象站。它們的尺寸、座向、建造的材料、架構、機械設備以及控制的系統完全相同。

雙福品種之番茄在以泥碳土、蛭石及發酵豬糞堆肥混和所配製而成的盆栽介質中，比在其他盆栽介質中的發育良好，待幼苗長出第四真葉時，將之移植於15公分直徑的塑膠盆中，然後轉移至兩棟雙層塑膠溫室及室外，每一環境下，每次實驗各置500盆；兩棟溫室中，有一棟的相對濕度較高，稱之為A溫室；相對濕度較濕者，稱之為B溫室。冬天時溫室內的溫度比室外高；而夏天時，室內則比室外低溫；在夏天時，溫室內的溫度和光線強度呈顯著($p=0.05$)相關，如遮陰網蓋住A溫室後，在夏天可使室內溫度低至26.1℃，而室外平均為32.2℃。番茄在A溫室中生長旺盛，而且株高在冬季栽種者及夏季栽種者均比B溫室中及室外的番茄高，而且A溫室中的番茄葉片之大小及數目均比B溫室及室外者大及多；雖然番茄的著果數比室內者多一倍，但單一果實的重量，則是A溫室中者比室外者重66%。當番茄生長過程中，於冬季時，在室外的番茄普遍地罹患灰黴及菌核病，但室內的番茄卻未罹患菌核病;百粉病全年均可發生在室內及室外的番茄上，但在夏天的病情會比冬季時嚴重。*Phytophthora capsici*可以造成番茄的根及葉腐，它在夏季時比冬季時容易感染植物，在A溫室中，當此病原感染番茄之先，分別噴灑15, 16, 及24號分離株之拮抗細菌，可以顯著地（$p=0.05$）有效防治此病原引起之病害，此防治效應在溫室中比室外的環境下明顯。

雙層塑膠溫室可提供許多花卉作物有一穩定合宜的生長環境，這種性質可使生產者獲得高品質的切花及盆花產品。

在遮陰的溫室中，生長速率良好的花卉作物有麥桿菊、孔雀菊、鳳仙花、四季海棠、百合、波斯菊、萬壽菊、松葉牡丹、一串紅、大理花、康乃馨及翠菊;而在無遮陰的溫室中可生長良好的植物有雞冠花、百日草、波斯菊、百合、萬壽菊、松葉牡丹、視覺辣椒、康乃馨及翠菊。

依產生的花器數目而論，孔雀菊、鳳仙花、四季海棠、百日草、波斯菊、百合、非洲菊、萬壽菊、一串紅、及大理花，在有遮陰的溫室中，可產生較多的花朵;而雞冠花、大理花、視覺辣椒、康乃馨及翠菊在無遮陰的溫室中，比有遮陰的環境下，產生較多的花朵。

在有遮陰的溫室中孔雀菊、四季海棠、波斯菊、百合、非洲菊、一串紅、大理花、及翠菊等作物的花朵較大;而在無遮陰溫室中，能長出較大花器的植物有鳳仙花、雞冠花、百日草、松葉牡丹及康乃馨。

八、英文摘要：

Double-layer greenhouses are successfully used in North American area for over twenty years already for their considerable saving characteristics on energy consumption. In hot and humid subtropical area, it will be adopted and than further adopted to be a cooling as well as anti-typhoon greenhouse.

Initiate the adaptation of air-inflated double-glazing greenhouse and establish the construction, automation and operation technologies on engineering systems especially focused on cooling and anti-typhoon issued.

Two double-polyethylene air-inflated modular greenhouses, which are able to withstand the impact of strong wind, equipped with fan and pad cooling system were built. A weather station and a data acquisition system has been established to monitor the relevant environmental variables necessary for analysis. In the first year, these two double-layer greenhouses were operated in the manner which were empty interiorly with different created environment with respect to the result of different control modes provided by the mechanical facility and electrical control system. The data collected were compared for these two completely same greenhouses.

In the second year, experimental work was continued carrying out in the double-polyethylene air-inflated greenhouses, in which a year-round cropping system was planted under different control models.

A dynamic root floating hydroponic system was mounted and then started planting leafy vegetables, tomato and cucmber under air-conditioning control.

The design of original two double-layer greenhouses were corrected. Another two greenhouses having the same construction based on the improved design were built in Taichung District Agricultural Improvement Station. The necessary weather station was also established to measure climatic parameters.

In the third year, the mechanically improved double-layer greenhouse systems were continuously doing experiment in cooperated with the hydroponic system to understand the extent of Improvement of living environment on plant especially on the cooling issue.

In the summer of 1989. Taiwan area was threatened several times by the typhoon alarms. Fortunately only Typhoon Sarah, a mid-sided typhoon, attracted Taipei on September 11. No damage reported on our greenhouses. In the past seven years, all the four greenhouses are still intact and function well.

Tomato seedlings(cv. Double Fortune) developed quite well in potting mix (mixture of vermiculite, peat, and composted swine manure) as compared to growth from other

container media. Following transplantation, 3,000 tomatoes were cultivated in two double -layered plastic greenhouses and an open field. Greenhouse A possessed a higher relative humidity than those of greenhouse B and open field. The temperatures in both greenhouses were higher in winter and spring and lower in summer than outdoors. The temperature significantly (p=0.01) correlated with the light intensity. When sun screen was covered on greenhouse A, the temperature was reduced to 26.1℃ compared to 32.2℃ in the field in the summer. Tomatoes grew much vigorously and higher in greenhouse A than in greenhouse B and outdoors. Tomato plants in greenhouse A had a larger leaf size and more number of leaves than the plants growing in greenhouse B and outdoors. Although the number of fruit setting on the plants at outdoor was one time more than the plant growing in the greenhouses, fruit weight from the plants growing in greenhouse A was 66% heavier than from the plants growing at outdoor. Various diseases occurred on tomatoes during its growing stage. Gray mold, Sclerotinia stem and fruit rot occurred rather frequently in the field in winter. Whereas, no Sclerotinia rot was found in greenhouses. Powdery mildew occurred all year-round in greenhouses and field. This disease, however, became severe during the summer time. Phytophthora capsici caused root rot and leaf blight of tomatoes and was applied as a model of disease control. This pathogen infected plants more readily in summer than in winter. Applying antagonistic bacteria (isolates #15, #16, #24) was able to significantly control the pathogen(p=0.05) in greenhouse A. The efficiency of disease control apparently became more pronounced in greenhouse than outdoors.

In summary, double covered plastic houses provided an consistent and suitable environment for the growth of many flower plants. This controlled environment could allow farmers to grow high economic flower plants such as cut flowers and thus produces high quality and uniform productions.

Plants having good growth rate inside the shaded greenhouse were straw flowers, French marigold, impatiens, begonias, lilies, cosmoses, African marigolds, rose-mosses, scarlet sages, dahlia, carnations and China asters. Plants grown good inside unshaded greenhouse were cockscombs, zinnias, cosmoses, lilies, African marigolds, rose-mosses, floss flowers, ornamental peppers, carnations and China asters.

If the research based on the consideration of flower number, the plants including French marigolds, imapatien, begonias zinnias, cosmoses, lilies, gerberas, African marigold, scarlet sages and dahlias could be produced many flowers inside the shaded greenhouse. In the mean while, the plantes such as French marigolds, impatiens,

cockscombs, zinnias, cosmoses, lilies African marigolds, scarlet sages, dahlias, ornamental peppers, carnations and China aster also could produce a lot of flowers inside unshaded greenhouse.

The flower sizes were found larger in shaded greenhouse, if French marigolds, begonias, cosmoses, lilies, gerberas, African marigolds, scarlet sages, dahlias and China aster were planted. In additions, the flower sizes were also large in unshaded greenhouse when French marigolds, impatiens, cockscombs, zinnias, cosmoses, lilies, gerberas, African marigolds, rose-mosses, dahlias, carnations and China asters were planted.

九、已發表報告目錄

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2. Wu, C. H., Chang H. S., and Shaw J. T. 1990. Evaluation of Plant Microclimate in semitropical Greenhause systems. ASAE paper No.904536, Chicago, IL, U.S.A.
3. 吳中興. 1991. 充氣式雙層塑料溫室本土化研究。台灣地區環控農業機械與工程研討會。台北市。
4. Wu, W. S., and Wu, C. H. 1992. September. Effects of Double-layered Plastic Greenhouse on Cultivation Tomatoes. Memoris of yhe College of Agriculture. National Taiwan University, Vol. 32, No. 3.
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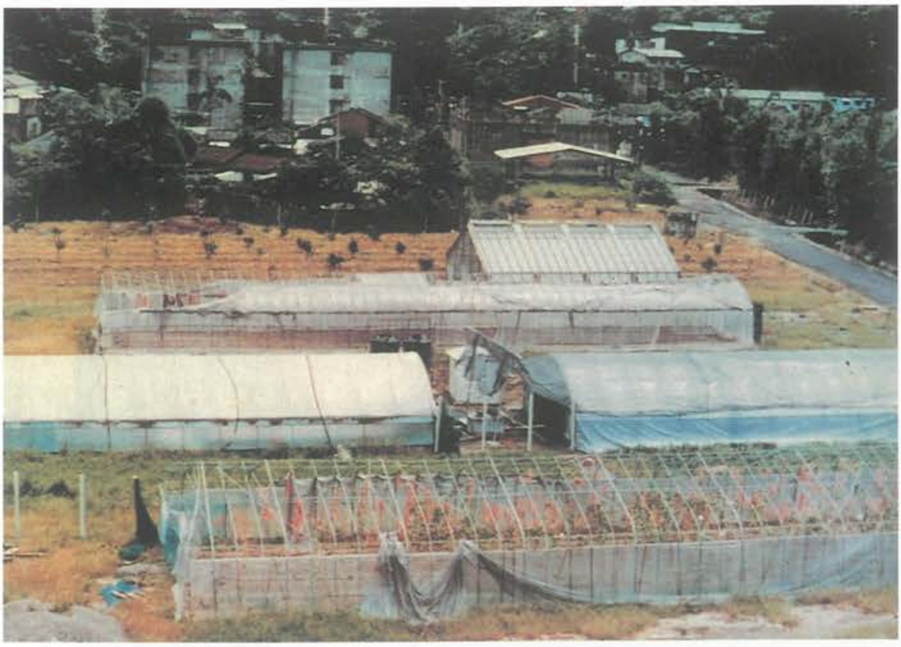
6. Wu, C. H., Chang, H. S., Kao, T. C., and Wu, W. S. 1993. June. Environment-Controlled Hydroponics inside Subtropical Air-Inflated Greenhouse. ASAE Paper No. 934046, Spokane, WA. U.S.A.
7. Wu, C. H., Hu, S. W., Wu, W. S., and Lu, J. S. 1993. June. Fuzzy Related Computer Vision for Diagnosing Tomato Diseases. ASAE Paper No. 933033, Spokane Washington, U.S.A..

圖一、第一年新建完成之雙層塑料溫室外觀

Fig. 1. The double-layer greenhouses newly constructed in the first year.

圖二、第三年夏天使用中的雙層塑料溫室外觀

Fig.2. The outlook of greenhouses in the summer of the third year.



圖三、雙層塑料溫室兩側的其它溫室，均在楊茜颱風中重創

Fig. 3. Neighboring greenhouses were seriously damaged by typhoon

圖四、位於雙層溫室旁邊被颱風吹毀之溫室

Fig. 4. A neighboring greenhouse destroyed by typhoon

一、計畫名稱：

中文：台灣人工種子技術之開發

英文：Development of artificial seed technology in Taiwan

二、執行期限：1990年7月1日至1994年6月30日

三、執行單位：財團法人生物技術開發中心

四、美方合作單位及人員：美國農部，

五、中方研究人員：宋台生、滕蕙蘭、劉燕君、蔡瑜卿（林喬柏）

六、計畫目的：1.建立萵苣與胡蘿蔔的液態組織培養系統

2.利用醱酵槽大量繁殖體胚

3.開發理想的體胚包埋材料及包埋程序，使人工種子得以在長期保存後仍具備高度發芽率及幼苗成活率

4.發展人工種子的自動化生產設備

七、執行成果摘要：

本實驗之目的在於研究2公升容積之生物反應器進行萵苣細胞懸浮培養放大過程的研究。影響細胞生長及分化之因子，包括泡沫形成，槽壁效應，通氣量及溶氧率。槽壁效應結果在24小時培養內嚴重損失10％之接種源，而使得降低不定芽再生率。引起槽壁效應之因子可能有二：1)泡沫形成與氣泡及接種源有關，2)通氣產生泡泡。接種源經400 micron之過濾網過濾後再經蒸餾水清洗，可避免泡泡形成，因可除去單一細胞及細胞殘留物。於生物反應器中央置放一150 micron之過濾槽體將接種源及通氣隔離可降低因氣泡引起之槽壁效應。克服槽壁效應之後，2公升之生物反應器培養之不定芽再生率顯著提高與以125 ml之三角瓶經1-2 vvm通氣量培者相似。反應器培養結束時其溶氧率大約70-80％。

八、英文摘要：

A scale-up process of lettuce suspension culture in a bioreactor with 2 liters working volume was investigated. Factors that influenced cell growth and differentiation were tested, including foam formation, wall effect (inoculum adhering onto the vessel wall above the medium level), aeration and dissolved oxygen (DO). The wall effect resulted in severe loss of inoculum-10 % within 24hr. Loss of inoculum caused a significant decrease in shoot regeneration. It was identified that the wall effect was caused by two factors: 1) the foam generated from the interaction between air bubbles and inoculum, and 2) the bubbles produced by aeration. The foam generation could be prevented by sieving the inoculum through a 400 μ m screen filter and then thoroughly rinsing with distilled water. This was to remove single cells ; cell debris and contents of broken cells. The wall effect caused by air bubbles could be prevented by putting a 150 μ m screen

column in the center of the bioreactor vessel to isolate the aeration area from the inoculum. After the wall effect was removed, shoot regeneration in the 2-liter bioreactor increased significantly to a level similiar to that in 125 ml flasks at an aeration rate of 1-2 vvm. The DO level for such shoot regeneration level was around 70-80% at the end of bioreactor culture.

九、已發表報告目錄：

1. Teng W. L. and Liu Y. J. 1993. Restricting hyperhydricity of lettuce using screen raft and water absorbent resin. Plant Cell, Tissue and Organ Culture 34:311-314.
2. Teng W. L. and Liu Y. J. and Soong T. S. 1992. Rapid Regeneration of Lettuce from Suspension Culture. HortScience 27(9):1030-1032.

一、計畫名稱

中文：少量有機添加物防治*Rhizoctonia solani*引起苗猝倒病之探討

英文：Biological control of *Rhizoctonia solani* with minimal organic amendment

二、執行期限：1987年7月1日至1990年6月30日

三、執行單位：國立台灣大學植病系

四、美方合作單位及人員：

Biocontrol of Plant Disease Laboratory

USDA, ARS, Beltsville Area

Dr. Robert D. Lumsden

Department of Plant Pathology

University of Hawaii

Dr. Wen-hsiung Ko

五、中方研究人員：莊再揚教授

六、計畫目的：

1. 篩選適當的有機添加物與拮抗菌組合，以抑制土媒病原菌。
2. 測定上述有效組合對苗猝倒病的防治效果。
3. 探討有效組合的防病機制。

七、執行成果摘要：

土壤中添加10種有機物，測試其對*R. solani*厚膜胞子發芽抑制的影響，以濃度0.1%及0.5%的苜蓿和大豆殘體的效果最好。蔗渣與雞糞以10:1比例混合亦有相同的抑制效果。此類有機添加物與拮抗菌混合能有效減少*R. colani*所引起蘿蔔幼苗猝倒病。此種抑病效果可能由於處理土壤產生揮發性氣體和有毒物質所致。由拮抗菌所產生的有毒物質顯著受pH值及基質影響。當有效的有機添加物－拮抗菌組合加入土壤中後種植蘿蔔，在第一次種植時，苗猝倒病會稍微增加，以後連續種植時，病害迅速下降。拮抗菌製成丸粒製劑的防病效果較單獨使用菌絲為佳，而丸粒中的有機質種類與濃度會影響防病效果。雖然土壤中添加少量有機物混合拮抗菌的防病效果會因土壤種類不同而有所差異，但仍具有防治土媒病害的實用潛能，惟需對微生物在土壤中的生態關係做更詳細的了解。此外，由Dr. Lumsden 所贈送在美國有效的拮抗菌，在本試驗中對*R. solani*所引起的苗立枯病並無防治效果，此表示生物防治所使用拮抗菌會有地理性的限制。

八、英文摘要：

Different soils were amended with alfalfa meal, bagasse, chicken manure, oilseed cake, soybean waste, rice hull, mushroom compost, corn cob, green peanut hull, roast peanut hull and crab to test the germination of monilioid cell of *R. solani* on the soil

surface. Results showed that alfalfa meal and soybean waste at the concentration of 0.1% and 0.5% were more effective to inhibit monilioid cell germination. The mix of bagasse and chicken manure at a ratio of 10:1 also showed the similar effect. The combinations of organic matter with antagonists were able to reduce *Rhizoctonia* damping-off of radish seedlings. This suppression might partly due to volatile substance evolved from the amended soil, and partly due to the production of toxic substance. The production of toxic substance by the antagonists might be greatly influenced by the pH value and substrates used. In greenhouse test, radish damping-off caused by *R. solani* was slightly enhanced at the first planting and then decreased after repeated planting when effective combinations of organic matter with antagonists were added into infested soil. These combinations were also not effective to suppress the disease in the first planting in field test. Alginate pellets were better than mycelia alone to suppress the disease. The efficacy of suppression was mainly depended on quality and quantity of organic amendment. Although the efficacy of disease suppression was inconsistent and might vary from soil to soil, biological control of soil-borne plant pathogens by the combination of antagonist with minmal organic amendment is still promising. However, it needs to understand more things about the ecological relationship of microorganisms in soil. In addition, biocontrol agents obtained from Dr. R. D. Lumsden were not effective to control *Rhizoctonia* damping-off in present experiment, indicating use of biocontrol agents may have some geographical limitation.

表一、拮抗菌混合大豆殘體對立枯絲核菌所引起蘿蔔苗猝倒病之防治效果。

Table 1. Efficacy of mix of antagonist and soybean waste on control of radish damping-off caused by *Rhizoctonia solani*

Antagonist isolate	Percent disease severity in successive replantings		
	1st	2nd	3rd
0.5% Soybean waste			
#32	73.3 ab	47.8 bc	7.8 b
#56	80.0 a	81.1 a	60.0 a
0.1% Soybean waste			
#32	70.0 ab	23.3 c	3.3 b
#56	77.8 a	73.7 ab	17.8 b
Conrol	63.3 b	73.3 ab	56.7 a

表二、拮抗菌 *Trichoderma viride* 之菌絲、厚膜胞子或分生胞子混合不同有機質所製成丸粒對防治立枯絲核菌所引起蘿蔔菌猝倒病效果之變方分析。

Table 2. ANOVA of mycelia chlamydospores and conidia of *Trichoderma viride* mixed with organic matter in alginate pellet for control of radish damping-off caused by *Rhizoctonia solani*.

Sources	DF	Mean square	F value	P
Treatment [1]	2	315.47	19.89	*** [4]
Inoculum conc. [2]	1	3460.49	218.21	***
Organic matter [3]	3	19.75	1.25	0.324
Organic matter con	1	250.03	15.77	***
Inoculum conc. X organic matter	3	66.34	4.18	*
Organic matter X organic matter con	3	19.01	1.2	0.34
Inoculum conc. X organic matter con	1	16.21	1.02	0.326

1 Treatment: mycelia, chlamydospores and conidia are prepared as alginate pellet.

2 Inoculum concentration: 0.1% and 0.5% *Rhizoctonia* infested soil.

3 Organic matter: alfalfa meal, soybean waste, corn cob and crab shell at the contration of 0.1% and 0.5%.

4 *, **, and *** are significantly at 0.05, 0.01 and 0.001 levels, respectively.

一、計劃名稱：

中文：血清學探針之開發用於柑桔病原之檢驗及標誌探針應用於探測病原感染及傳播。

英文：Development of serological probes for detection of citrus pathogens and application of labeled probes for measuring pathogen infection and dispersal

二、執行期限：1988年7月1日至1997年6月30日

三、執行單位：國立台灣人學植物病蟲害系

四、美方合作單位及人員：

Horticultural Research Laboratory, USDA-ARS Orlando, Florida, U.S.A.

Dr. Stephen M. Garnsey and Dr. Tim R. Gattwald

五、中方研究人員：蘇鴻基

六、計劃目的：

研發柑桔黃龍病(立枯病)擬原核細菌及重要病毒之診斷用血清及核酸探針而應用於病原之感染傳播生態研究及檢疫，進而利用於無毒種苗之生產。

七、執行成果摘要：

分離並比較探討柑桔萎縮病毒(citrus tristeza closterovirus=CTV)之三種不同系統包括分別對柚類、甜橙及寬皮柑引起嚴重莖陷及萎縮病徵之系統，以及苗黃系統(SY)。SY系統普遍分佈於台灣及其他亞洲國家，一般感染甜橙及寬皮柑類無病徵，但不永續於柚類。柚莖陷系統(Pum/SP)首次發現於台灣，稱為萎縮系統(CTV-D)，近年來普遍發生，對感染油、葡萄柚及萊姆引起嚴重萎縮及莖陷，在寬皮柑中複製低，在檸檬不引起苗黃病徵。橙莖陷系統(SwO/SP)在台東發現，在印尼及華南存在，引起寬皮柑嚴重莖陷之系統(Mand/SP)發現於馬西亞及泰國之寬皮柑類。與美方共同開發多種單元抗體，用於鑑別CTV系統，單元抗體3E10判認一般抗原基對所有系統反應，而10E3單元抗體對弱毒系統不反應，用於交叉保護效果之判斷，4E12 單元抗體對SY系統及Mand/SP系統反應。

柑桔黃龍病的病原擬細菌在主體內的濃度低且分布不平均，嘗試開發單元抗體做為病害之診斷探針，但因偵測結果不夠穩定而無法有效應用，為了研製效果穩定且特異性與敏感度高的病原偵測採針，經基因選擇，製備出核酸探針以供病害診斷及病原檢疫之應用。以質體pBS為載體，將病原核酸片段載於其上，由其形成的836個質轉殖株(Transformants)中找到3個含有病原特異性核酸片段的質體轉殖株，經重覆確認其特異性後，選其中最穩定者來標誌成一個核酸探針。此黃龍病 原核酸探針的點漬雜配法[Dot hybridization (DH)]偵測效果穩定且其特異性與敏感度高，因而將之選定為核酸探針1(DNA probe 1)，廣泛應用於病原之偵測及生態之研究。以DH 法檢驗柑桔病株中GO病原在椪柑、桶柑、柳橙及柚類可疑之感病株，均可檢出GO病原感染而健株及根腐或營養缺乏呈黃龍病相似病徵之病株中均測不到GO、證實此DNA

探針對此病原有專一性。經乾燥之病組織比新鮮組織之DH測定效果為高。經生物技術基因選殖之核酸探針，經解明病毒核基因體序列而研製不同引子對(Primer pairs)，利用於建立PCR技術，應用於高敏感度之黃龍病病原偵測。

柑桔黃龍病原(Greening organizm=GO)之核酸探針偵測方法改良上，核酸純化的過程已從12步驟簡化為5步驟。PCR法發現將黏合溫度由46℃調高至56℃時效果最佳，並可避免非特異性片段的產生。由椪柑病株選殖到第二片GO特異性的核酸探針(DNA probe 2, 1.1 Kb)，經定序訂出第二引子對，行PCR測試發現此探針對台灣嚴重病徵分離病株有強增幅反應，但和馬來西亞沙勞越及日本硫球的分離株則不反應，而沙烏地阿拉伯的分離株則又呈現正反應。此第2對引子對之特異性可利用於GO系統分離株之鑑別。從田間外表健康之桶柑株以敏感度高之PCR法偵測到病原潛伏感染，所得無病徵分離株嫁接試驗，超過年半仍未呈病徵，始終…偵測到低濃度GO，初步認定為弱毒系統，進一步交叉保護在實驗中。偵測田間椪柑及桶柑中GO與萎縮病毒(CTV)複合感染的相互作用證實在黃龍病原感染的初期潛伏期中，GO抑制CTV病毒複製，比單獨感染株為低。經黃龍病柑桔病芽嫁接證實烏柑仔(*Severinia buxifolia*)及木蘋果(*Limonia acidissima*)，可能為新中間寄主。

八、英文摘要：

A comparative study was made on the three distinct CTV strains causing severe stem -pitting (SP) and stunting, respectively, in pummelo, sweet orange and mandarin trees, and an ordmary seedling-yellow (SY) strain. The ordinary SY strain caused symptomless infections in sweet orange and mandarin trees, but did not persist in pummelo trees which are grown widely in Asian countries. The pummelo SP strain (Pum/SP), called dwarf strain of CTV(CTV-D), has become common infection in pummelo trees in recent years. The Pum/SP replicated well and caused severe dwarfing and SP in pummelo, grapefruit, and lime trees. It replicated poorly in mandarin, and did not induce seedling yellows symptoms in Eureka lemon. The sweet orange SP strain (SwO/SP) found in eastern Taiwan, Indonesia and southern China, caused sever SP and moderate stunting in sweet orange trees and SY symptoms in Eureka lemon, but did not cause any symptoms in mandarin and pummelo trees. The mandarin SP strain (Mand/SP) found in Malaysia and Thailand caused sever SP in mandarin. Three monoclonal antibodies (MAB) were prepared through binational collaboration for differentiating CTV strains. The MAB 3E10 recognized the epitope common to all isolates, while 10E3 using for evaluating cross protection, did not react with the mild strain, and 4G12 did not react with SY and mandarin SP strains.

Diagnostic McAbs against greening (Likubin) pathogen was prepared, however very

low value of ELISA test with the MABs was demonstrated. The fact that McAbs did not show constant reaction in ELISA test, was presumable due to low content of greening organism (GO) in the infected citrus tissues. Preparation of sensitive DNA probes showing highly specific to GO in dot hybridization test, was developed through screening from 836 transformants. Considerable sensitivity of dot hybridization blotting with present labeled DNA probes was demonstrated in detection of GO-DNA in crude extract of disease tissues from the all citrus commercial cultivars. Drying treatment of disease tissues increased sensitivity of dot blotting by improving the extraction of GO- DNA. Through sequencing DNA probes, adequate primer pairs were derived for developing polymerase -chain reaction (PCR) technique wich was demonstrated to be much high sensitive in GO detection. In improving trial of GO (greening organism) detection, DNA extraction procedure was simplified from 12 steps to 5 steps, and annealing temperature was changed from 46℃ to 56℃ in PCR test for obtaining best amplification of target DNA without amplification of non- specific DNA bands. The second DNA probes (1.1 Kb) was cloned from DNA extract of GO-infected Ponkan tree, and the second primer pairs were syntheaized through sequencing the DNA probe 2. Amplification detection of GO-DNA by PCR with the 2nd primer pair revealed strong reactions with GO- DNA template of Taiwanese severe isolate and Saudian isolate, while no amplification was demonstrated with Sararawak isolate of Malaysia and Okinawa isolate of Japan. The 2nd primer pair might be used for differentiation of GO strain isolates. Some mild GO isolates showing symptomless in inoculation test within one and half years, were isolated from healthy-looking citrus old trees in the field. The mild isolates have been subjected to cross- protection trial which is under way. Go infection showed interference with citrus tristeza virus (CTV) by suppressing virus replication in citrus trees in the field. Chinese box orange (*Severinia buxifolia*) and wood apple (*Limonia acidissima*) were found to be the new alternative hosts of GO.

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一、計畫名稱：

中文：建立甘藷病毒標準檢定制度

英文：Development of a standard procedure for sweet potato virus indexing

二、執行期限：1987年7月1日至1991年6月30日，共4年

三、執行單位：亞洲蔬菜研究發展中心，國立中興大學遺傳工程中心

四、美方合作單位及人員：Suzanne S. Hurtt

五、中方研究人員：S. K. Green, 徐堯煇

六、計畫目的：

1.分離台灣甘藷常見之病毒，製造檢測用抗血清及單株抗體。

2.病毒對甘藷產量之影響。

3.建立甘藷病毒標準檢定制度，以確保國際種源交換時之安全性。

七、執行成果摘要：

亞蔬中心於1985年受植物遺傳資源國際理事會指定為東南亞及太平洋地區甘藷種源中心。因為甘藷多為以蔓或貯藏根進行營養繁殖，所以目前幾乎所有種源都感染病毒或菌質。在甘藷上常見的病毒包括甘藷羽狀斑駁病毒 (SPFMV，分佈全世界)，甘藷微斑駁病毒 (SPMMV，東非)。 甘藷黃化萎縮病毒 (SPYDV)，甘藷病毒II (SPV-II)，甘藷潛隱病毒(SPLV)和甘藷捲葉病毒 (SPLCV) 僅在台灣發現。此計畫的目的就是確保國際種源交換時為無病毒狀態。

在此試驗中，使用熱處理、化學處理及莖頂培養以減少病毒。血清學的方法，例如ELISA分析、免疫電子顯微鏡觀察及嫁接到指示植物，以評估檢測病毒的有效性。SPFMV、SPYDV、SPLV、SPV II之病毒經純化後，製造其抗血清，SPMMV之抗血清購自英國，SPLCV則以核酸探針進行測試。經過莖頂培養前兩個月，30公分的莖頂先經過熱處理 (37℃，60/RH，3.1 Klux/12 hrs) ，可有效地達到去除病毒的效果。若在培養基中加入30-100 mg/l之病毒抑制劑，對去除病毒沒有任何影響，但當抑制劑濃度超過50 mg/l 時，會產生嚴重之藥害。

亞蔬中心已發展出甘藷病毒去除及檢定方法。其方法如下：經過熱處理後進行莖頂培養，然後進行2次ELISA試驗，測試 SPFMV、 SPYDV、SPMMV、SPLV和SPV-II病毒。第一次ELISA (E1) 在莖頂培養後4-5葉之苗期進行。第一次ELISA測試為負反應的植物，切除1公分之莖頂，至長到苗期後，進行第二次ELISA試驗 (E2)。同時，以核酸雜合法 (ITB) 測試 SPLCV。第一次ELISA測試為負反應之植株至長大後，進行兩次嫁接(G1，G2) 以確定沒有其他病毒。僅有E1、E2、ITB1、ITB2、G1、G2都為負反應的植株才留下來，以此流程，共獲得322個無病毒品系。

9個無病毒的育種品系在田間生長，其產量與未經莖頂培養的產量相比較，結果顯示經過莖頂培養後，在第2、3、4 個生長季，其產量高於未經莖頂培養之田間繁

殖株。即使經過莖頂培養的植株在田間已再感染病毒，在第4個生長季其產量比未經莖頂培養的營養繁殖株高出25% 到211% 之間。

八、英文摘要：

In 1985 AVRDC was officially designated by the International Board of Plant Genetic Resources (IBPGR, now IPGRI) as the field genebank for sweetpotato germplasm for Southeast Asia and the Pacific. Since this crop is almost entirely vegetatively propagated by the use of vine cuttings and storage roots, almost all germplasm is known to be infected with viruses and mycoplasma-like organisms (MLO). The most common virus reported from sweetpotato is feathery mottle virus (SPFMV) which occurs world-wide. Sweetpotato mild mottle virus (SPMMV) has only been reported in East Africa. Sweetpotato yellow dwarf virus (SPYDV), sweetpotato virus II (SPV-II), sweetpotato latent virus (SPLV), and sweetpotato leafcurl virus (SPLCV) have so far only been found in Taiwan.

This project was initiated to facilitate international movement of the mainly vegetatively propagated germplasm by ensuring their disease-free status.

Thermotherapy, chemotherapy, and meristem-tip culture were tested for their use in virus elimination. Serological methods, such as ELISA, and immuno tissue blotting elect-ronmicroscopy, as well as graft indexing to susceptible indicator hosts were tested to assess their effectiveness in detecting viruses.

Following virus purification, various polyclonal antisera against FMV, SPYDV, SPLV, and SPV-II were produced. A nucleic acid probe was developed for the detection of SPLCV, a germinivirus. SPMMV antiserum was obtained from A.T. Brunt, U.K.

Meristem culture was found to be very effective in eliminating viruses. Virus elimination efficiency ranged from 75-80% in different cultivars.

A two-month pre-meristem excision heat treatment of 30-cm-long stemtips at 37℃ (60/RH, 3.1 klux/12 hrs photoperiod) was found to significantly increase the percentage of virus elimination. However, placing the meristems on media containing the antiviral agent virazole at concentrations ranging from 30-100 mg/l had no effect on virus elimination. Phytotoxicity was severe at concentrations above 50 mg/l.

The following virus elimination and virus indexing methods was developed for routine use at AVRDC. Meristem tip (0.3 mm) culture of heat-treated stemtips followed by virus indexing by two ELISA test using antisera to FMV, SPYDV, SPMMV, SPLV, and SPV-II. The first ELISA test (E1) is done on tissue cultured planlets at the 4-5 leaf stage, the second ELISA test (E2) on tissue cultured plantlets derived from 1 cm stemtips of E1

-negative plants. Simultaneously with the ELISA tests, an immuno tissue blotting test (ITB) is conducted for the detection of SPLCV. Two graft indexings (G1, G2) are also performed on mature plants derived from the remaining portions of the E1-and E2 negative plantlets for the detection of unknown viruses, for which no antisera/nucleic acid probes are available. Only plantlets derived from E1-, E2-, ITB-, G1-, and G2-negative plants are kept and propagated for distribution.

A total of 322 virus-free lines were obtained by this method.

Nine virus-free AVRDC breeding lines were grown for 4 growth cycles in the field and their yields were compared with yields from field propagated clones of the same lines. Significantly higher yields were obtained from meristem- derived plants in the second, third, and fourth growing cycles in the field. In the fourth growing cycle, yield increase of meristem-derived lines over field propagated lines ranged from 25 to 211%, depending on the line, even though virus reinfection of most meristem-derived plants had already reached that of field propagated plants.

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一、計畫名稱

中文：利用分子標誌選育多重抗病毒之熱帶番茄品種之方法研究

英文：Methodology development of multiple virus resistance in tropical tomatoes using molecular markers

二、執行期限：1993年7月1日至1998年6月30日，共5年

三、執行單位：亞洲蔬菜研究發展中心，國立中興大學植病系

四、美方合作單位及人員：

美國康乃爾大學

S. D. Tanksley & D. Bernacchi

五、中方研究人員：S. K. Green, C. G. Kuo, P. Hanson, 葉錫東

六、計畫目的：

發展分子標誌檢視抗病基因，以加速選育抗多重病毒之熱帶番茄品種

七、執行成果摘要：

番茄黃捲葉病 (TYLCV)，胡瓜嵌紋病毒 (CMV)，西瓜銀斑病毒(WSMV)，馬鈴薯Y病毒 (PVY) 是危害熱帶及亞熱帶番茄主要的病毒病害。目前尚無抗單一或多種病毒之栽培品種，但已在野生種*L. chilense*(抗TYLCV) 與*L. hirsutum*(抗PVY，CMV，TYLCV) 發現具有抗這些病毒之特性。因此擬發展分子標誌檢視抗病基因，以加速將抗病基因導入耐熱，耐細菌性萎凋及抗番茄嵌紋病毒之亞蔬栽培品種。有關西瓜銀斑病毒之抗病篩選，應用含有N-protein基因之農桿菌菌株對植物進行轉化作用，目前已獲得含有N-protein基因之轉型植株，將利用引子檢視分離族群中是否含有N-protein基因。

以野生種*L. chilense*和*L. hirsutum*分別作為抗TYLCV和CMV之抗病來源，然後對後代族群進行抗病篩選。一旦抗病性固定，將以90個以上涵蓋所有染色體之分子標誌進行RFLP分析。目前TYLCV之抗病篩選已進行到B1F4，而CMV之抗病篩選進行至BC1F2。此抗病性的固定，目前仍繼續中. 以抗病植株與感病植株進行雜交，而其F2植株以分子標誌分析含有一個或多個，或是沒有基因導入。F2植株自交以產生F3植株，F3將進行抗病篩選以確定那些基因導入與抗病性有關。

此外，以*L. hirsutum*作為抗病來源之抗TYLCV以及抗PVY品系 (*L. esculentum*)，進行RFLP分析那些*L. hirsutum*基因與抗病性有關。抗PVY之*L. esculentum* CLN 808分析結果顯示，已在第4，7，9，10對染色體有*L. hirsutum* 基因之導入，抗TYLCV之*L. esculentum* H 24分析顯示在第8，11對染色體有*L. hirsutum*基因導入。這些基因的導入可能與抗病性有關。以抗病的親本與感病的親本進行雜交，其F2植株進行RFLP分析是否有基因導入。這些含有不同基因導入之F2植株，自花授粉以產生F3植株。F3植株進行抗病篩選，以確定那些基因導入與抗病性有關。在PVY之試驗，F3族群正進行抗病篩選，在TYLCV之試驗，F2植物正進行RFLP分析。

西瓜銀斑病毒之抗病篩選，是利用中興大學葉錫東教授提供含有N- protein基因之農桿菌菌株，對植物進行轉化作用，轉型植株應用PCR方法測試N-protein基因，並以西方墨點法測試基因的表現。其中一株R0植物，PCR方法檢測為正反應，但西方墨點法檢測為負反應。源自此R0之86株R1 植物，經西瓜銀斑病毒抗病篩選後，顯示57棵植株沒有病徵，且ELISA測試為負反應。這些植物已進行自交，並將進一步以PCR方法及接種試驗了解遺傳之穩定性。

八、英文摘要：

The four viruses TYLCV, CMV, WSMV and PVY, are major viral diseases of tomato in the tropics and subtropics. There is no resistance to these viruses, singly or combined, in horticulturally-acceptable tomato lines. However, resistances to CMV, TYLCV and PVY have been found in a few wild tomato accessions of *L. hirsutum* (PVY, CMV, TYLCV) and *L. chilense* (TYLCV). The identification of RFLP markers linked with these resistance genes would be useful to transfer all three resistances into improved AVRDC heat tolerant tomato lines which already carry bacterial wilt tolerance and tomato mosaic virus (ToMV) resistance.

This would ultimately reduce the time, cost, and unreliable virus screening/testings. In the case of WSMV, Agrotransformation with the N-protein construct was chosen and the inserted gene will be traced in segregating populations by use of primers flanking this gene.

For the introgression of TYLCV resistance genes from *L. chilense* and of CMV resistance from *L. hirsutum,* backcross lines were developed using the wild accessions as donors of resistance. Resistance will be fixed through repeated screening and generation advance. Introgressions common in all resistant lines will be identified by RFLP probing of the B1Fn (n = generation in which resistance is fixed) with >90 markers well distributed across all chromosomes. TYLCV crosses which involved embryo rescue for the development of F1 and BC1F1 have been advanced to B1F4 whereas CMV crosses are at the BC1 F2. Fixing of resistance in these crosses is still ongoing. Resistant backcross lines will be crossed with a susceptible parent, the F2 plants will be probed, and individual F2 plants with either none, single or multiple introgressions identified. These F2 plants will be selfed, the F3 families will be evaluated for disease reactions which will confirm which introgressions are essential for resistance.

A slightly different approach was chosen to map wild species introgressions in previously developed TYLCV and PVY resistant L. esculentum lines. Both resistances originated from different *L. hirsutum* accessions. RFLP probing was conducted on each

virus- resistant *L. esculentum* parent to identify *L. hirsutum* introgression possibly conditioning PVY or TYLCV resistance.

In the case of the PVY resistant *L. esculentum,* CLN 808, four introgressions on chromosomes 7, 9, 10 and 4 were found that may be associated with PVY resistance, where as in TYLCV resistant *L. esculentum* H 24, introgressions on chromosomes 8 and 11 were found that may be associated with resistance.

Each of the resistant parents were crossed to a susceptible parent and crosses were advanced to the F2. F2 plants were probed with RFLP's to identify plants carrying none, single or multiple introgressions. These selected F2 plants carrying different introgression combinations were self-pollinated to obtain F3 families. Disease reactions of the F3 families will be determined which will confirm the introgressions necessary for resistance. In the case of PVY, F3 families are presently being evaluated for disease resistance, and F2 plants of the TYLCV cross are undergoing probing.

For WSMV, agrotransformation with the N-protein gene (kindly provided by S. D. Yeh of NCHU) was performed and transformed plants were tested by Western (for expression of the gene) and by PCR for gene insertion. One R_0 clone (PCR positive, but Western negative) was further investigated. Eighty six R_1 plants of this clone were screened for WSMV resistance and 57 were found symptomless and ELISA negative. These plants were selfed and are now undergoing confirmation by PCR and by WSMV inoculation to determine stability of inheritance.

九、已發表報告目錄：

Green, S. K., and P. Hanson. 1996. A new source of PVS resistance. Rpt. Tomato Genetics Coop. Newsletter 46:9.

一、計畫名稱：

中文：臺灣作物根部疫病菌的抑病土壤測定與機制

英文：Detection and mechanisms of soils suppressive to root rot caused by *Phytophthora* species in Taiwan

二、執行期限：1989年7月1日至1993年6月30日，共5年。

三、執行單位：臺灣省農業試驗所嘉義分所

四、美方合作單位及人員：

Department of Plant Pathology, University of Hawaii, USA

Dr. Wen-hsiung Ko

五、中方研究人員：安寶貞

六、執行目的：

1. 篩選甜椒根部疫病、柑桔根部疫病、及其他作物疫病之抑病土壤
2. 抑病土壤之抑病機制探討

七、執行成果摘要：

利用胞囊發芽率之高低來檢定本省土壤對疫病菌 *Phytophthora capsici, P. palmivora,* 及 *P. parasitica* 之抑菌能力，在測定之 486 個土壤樣品中，分別發現 80, 113, 101 個土壤對上述三菌有強烈之抑菌能力（胞囊發芽率在 20% 以下），而其中185, 62, 105個土壤則對上述三菌具有強烈之導病性（胞囊發芽率在 80% 以上）。通常一個土壤樣品對一種疫病菌具抑菌性時，對另兩種疫病菌同時具抑菌性，相關係數(R)均在 0.84 以上，且極顯著。抑菌土壤與土壤之色澤、質地、地上部之栽培作物種類無關，疫病菌之抑菌土壤對其他種根部病原菌並不一定具抑制能力。但土壤之抑菌能力則與土壤之酸鹼值 (pH) 相關性極高。經分析發現本省疫病菌之抑菌土壤之機制共有三種：(1)、抑菌土壤之酸鹼值在 pH5.0 以下，此類土壤在調解酸鹼值高於 pH 5.5-6.5 以上時，均尚失對疫病菌胞囊發芽之抑制能力，但在經100℃高溫處理10 分鐘後並不失去抑菌能力，此類土壤經分析均含有較高之交換性鋁離子 (Al^{+3}, 濃度在 50 ppm 以上），而且經證實此類土壤之抑病機制確因含有鋁離子所致，大部份之抑菌土壤均為此類。(2)、少部份抑菌土壤為中性或弱鹼性，此類土壤經高溫處理後尚失抑菌性，但抑菌能力與酸鹼值無關，經分析此類土壤含有較高之有機質，磷、鉀、鈣、鎂含量亦高，土壤中微生物濃度亦高，土壤抑菌性與生物因子有密切關係。(3)、有二特殊土壤（土壤酸鹼值分別為 4.5 與 8.5）因鋅離子與銅離子含量高於100 ppm，亦具抑菌能力。利用胞囊發芽法篩選出之抑菌土壤一般均對甜椒疫病有強烈之抑病能力。

八、英文摘要：

When spore germination was used to assay pathogen suppression, soils suppressive

to *Phytophthora capsici, P. palmivora,* and *P. parasitica* were found to be widely distributed in Taiwan. Soil samples collected from the same general areas showed considerable variation in pathogen suppression. Among the 486 soil samples tested, the numbers of soil samples suppressive to the three species of *Phytophthora* (Germination rate < 20%) were 80, 113 and 101, respectively. While the numbers conducive (Germination rate > 80%) were 185, 62 and 105, respectively. Generally, the three species of *Phytophthora* showed similar pattern of sporangial germination in the same soil samples tested. Many soil samples were either suppressive or conducive to all these three fungi. Only a few soils supported high germination rate of one or two *Phytophthora* species and low germination rate of others. The correlation of sporangial germination among them were positive, high (r = 0.85 for *P. parasitica* vs *P. palmivora,* 0.84 for *P. parasitica* vs *P. capsici,* and 0.87 for *P. palmivora* vs *P. capsici,* respectively) and very significant (P< 0.001). The degree of pathogen suppression was not correlated with soil texture, soil color or vegetation. Soils with pH lower than 5.0 were more suppressive than those with higher pH, although a number of soils with pH lower than 5.0 were conducive as well as a few soils with pH higher than 7.0 were suppressive. The quadratic relationship between soil pH and sporangial germination of the three *Phytophthora* were correlated significantly (P<0.001) and highly (r = 0.65 for *P. capsici,* 0.66 for *P. palmivora* and 0.70 for *P. parasitica*). When the pH was adjusted to 6-8 most acidic suppressive soils became conducive, while those suppressive soils with pH original higher than 7.0 still kept suppressive after acidic treatment. Most of suppressive soils with pH lower than 5.0 remained suppressive to *Phytophthora* after being steamed at 100 C for 15 min, while most suppressive soils with pH higher than 7.0 became conducive. When 21 suppressive soils and 27 conducive soils were analyzed, the concentration of exchangeable aluminum ion in 15 of 16 acidic suppressive soils was higher than 100 ppm. However, the aluminum concentration in all of the 8 acidic conducive soils as well as other conducive soils studied was less than 50 ppm. Two suppressive soils (one with pH 4.5 and one 8.5) contend zinc and copper ions higher than 100 ppm, respectively. The rest 8 suppressive soils were alkaline with organic compounds higher than 4% and also contend higher phosphorus, potassium, calcium, and magnesium ions, ranging from 500 to 3000 ppm, respectively. When 50 or 100 ppm of Al^{+3} ($Al_2(SO_4)_3$) was added to conducive or semiconducive soils, the 5 soils with pH values less than pH 5.0, but not the 2 soils with pH values higher than 5.5, increased the ability in suppression of sporangial germination of *Phytophthora capsici* dramatically. Concentration of Al^{+3} ion equal or higher than 50 ppm was effective in

inhibition of direct germination of sporangia and realizing zoospores of the three *Phytophthora* species in 2% V-8 juice. Most pathogen-suppressive soils were also suppressive to pepper wilt caused by *P. capsici.* Most suppressive soils inactived sporangia of *P. capsici* added to soils as well as increased the degree of tolerance of pepper seedlings to the same pathogen.

Table 1. Germination of sporangia of three *Phytophthora* species on soils collected from various locations in Taiwan

% of sporangial [1] germination	No. of soils collected		
	P. parasitica	*P. palmivora*	*P. capsici*
0 - 10	86	95	69
10.1 - 20	15	18	11
20.1 - 30	8	10	11
30.1 - 40	22	21	17
40.1 - 50	31	33	17
50.1 - 60	56	46	30
60.1 - 70	58	101	52
70.1 - 80	105	100	94
80.1 - 90	99	48	145
90.1 - 100	6	14	40
Total	486	486	486

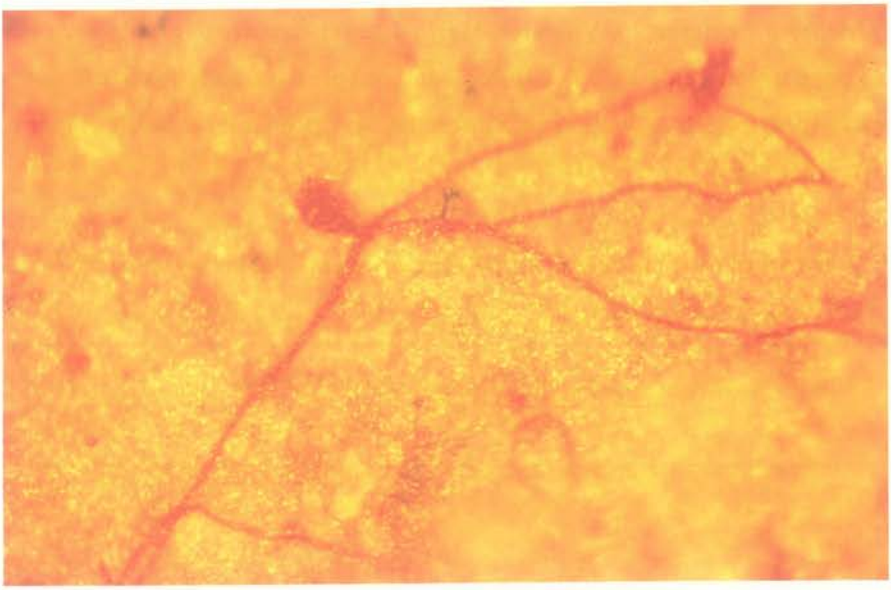
1. Soils supporting % of sporangial germination < 20% were considered as suppressive.

九、已發表報告目錄：

1. 安寶貞、柯文雄、高清文。1991。臺灣土傳疫病之防治及抑病土壤。植保會刊33: 142-147。

2. 安寶貞、柯文雄、高清文。1994。臺灣疫病菌抑病土壤之機制。植病會刊3:258-259 (摘要)。

3. Ann, P. J. 1994. Survey of soils suppressive to three species of *Phytophthora* in Taiwan. Soil Biol. Biochem. 26:1239-1248.



圖一、疫病菌胞囊在導病土壤上發芽4小時之情形。

Fig. 1. Germination of a sporangium of *Phytophthora palmivora* on a conducive soil block for 4hr.

圖二、疫病菌胞囊在抑病土壤上經發芽處理4小時後情形，原生質流失，或不發芽。

Fig. 2. Germination of sporangia of *Phytophthora palmivora* on a suppressive soil block for 4 hr.

圖三、甜椒幼苗在接種疫病菌的抑病土壤與導病土壤(各五個土壤樣品)上之存活情形。

Fig. 3. Comparison of sweet pepper seedlings grown on five suppressive soils and five conducive soils, respectively, artificially inoculated with sporangia of *Phytophthora capsici.*

一、計畫名稱：

中文：生物合理性化合物在害蟲防治之開發

英文：Development of biorational compounds for insect control

二、執行期限：1990年7月至1995年6月

三、執行單位：國立中興大學昆蟲系

四、美方合作單位及人員：

夏威夷大學昆蟲系

Franklin Chang

五、 中方研究人員：侯豐男

六、計畫目的：研究數種具安全性之生物合理性化合物在東方果實蠅及小菜蛾防治上之應用

七、執行成果摘要：

一種新型昆蟲化學不孕劑，J_{3230}，亦即 benzy-l,3-benzo-dioxole dcrivative(BBD)對東方果實蠅(*Dacus dorsalis*)生殖之效應，經用今此化合物之飼料餵飼幼蟲或成蟲來研究。以J_{3230}處理幼蟲後主要作用雄性成蟲，引起異常之精蟲生成作用，若此雄蟲與未處理之雌蟲交尾，則會降低卵之孵化。由檢視此雌蟲之貯精囊顯示在其內僅今少許精蟲，而雌蟲可接受以100ppm處理之雄蟲百比率只3.3%。處理後的卵切片圖顯示其內含均勻之卵黃並具核裂及壞死之卵內容物，此卵不發育。初羽化之雌雄蟲a 羽化後第1至9天餵予今960ppm J_{3230}之飼料，則所造成之不孕性主因處理雌蟲而非雄蟲所致。以電泳分析卵巢及卵內蛋白質，並未在受處理與未處理雌蟲發現其卵黃蛋白有明顯的差異，惟處理過的雌蟲稍為延遲蛋白質之合成。因此，J_{3230}似非對此果實蠅具有抗青春激素之作用。

另外，將老熟幼蟲曝於經此化學不孕劑處理之表土來探討對其生殖之效應，濃度在0.83-3.3mg/g下，則果實蠅之化蛹及孵化均顯著降低。羽化率由77%減至26%，處理後初期卵不孵化，但會逐漸恢復正常。濃度為1.66mg/g時，J_{3230}活性可在土中維持至少30天。當處理的土壤分別置於25℃、30℃或45℃下1小時、15天或30天，則活性會消退。上述置予25 ℃，1小時，孵化率會恢復43.57 到68%，處理15天，則恢復到59.77到85%，30天更會到67.85至 93%。此結果顯示J_{3230}之活性及持效性會因提高處理溫度及延長時間而降低。但其活性仍較在土中用丙酮處理者為高。若土壤曝於254nm紫外光15小時或366nm，48小時，則J_{3230}活性亦受抑制，顯示254nm紫外光破壞力較強。當J_{3230}加至殺菌後或未滅菌之土壤中分別為15天及30天，則兩者活性均無差異。以交叉交配處理過或未處理之雌雄蟲，試驗結果顯示J_{3230}主要影響雄蟲。由與處理過的雄蟲交尾之雌蟲貯囊內發現不含精蟲。雄蟲經J_{3230}處理後，其副腺之電泳圖不變，組織切片顯示處理及正常雄蟲精巢內含有正常的精囊細胞及精小胞，但精子束則含異常之鞭毛。因此J_{3230}乃會抑制雄蟲生殖細胞之微管系統。

有一種RH5992化合物係昆蟲脫皮素之非類固醇類似物會引起小菜蛾(*Plutella xylostella*)各幼蟲期之死亡率，但無法延長其發育。此化合物亦會減少幼蟲之食物消耗量，因而引起蛹重及成蟲壽命之降低，並減弱生殖力。但當卵以RH5992處理，則孵化率並未受影響。另以此劑滴定幼蟲並未顯著影響化蛹率及成蟲羽化率。

此RH5992係一種tebufenozide化合物，本試驗以甘藍葉浸漬不同濃度再餵飼各齡期小菜蛾，發現本劑僅在高於400ppm濃度處理各齡蟲，才會引起幼蟲死亡。雖然以400ppm處理1-3齡幼蟲，會引起100%之累積死亡率，但4齡蟲則僅為87.5%，用800ppm才能維持97.5%死亡率。對1、2、3齡蟲之LC_{50}。分別為96.3、95.3及105.8 ppm。對4齡蟲之LT_{50}為100ppm 3.4天，800 ppm 1.7天，但對其他齡蟲則分別為2.3天及12.3天。幼蟲之食葉量以100ppm處理者較對照者為低。當初孵化幼蟲連續飼養在用不同濃度RH5992處理之葉片上，其蛹重、羽化率、成蟲壽命及生殖力均會受影響。

昆蟲青春激素類似物(S-31183)，即pyriproxyfen，對小菜蟲蛾變態及對卵、幼蟲及蛹之毒性，加以測定。此劑對小菜蛾的卵具殺卵性，初產一天的卵較具感受性，而越老的卵，感受性越低。由組織切片顯示S-31183之主要作用發生在生產後12小時內，可破壞胚胎發育，引起卵黃消失，最後抑制孵化。施用S-31183會造成幼蟲死亡，對1-4齡蟲之LC_{50}分別為6.17、0.13、1.04及8.48ppm。此外，此化合物亦會引起幼蟲、蛹及成蟲之變態，而蛹期最具感受性。其LC_{95}只為0.02ppm。大部份蛹體受處理後均無法羽化，少數會羽化者，只進行半途而死，或形成異常翅型，無法移動、取食以及交尾。

八、英文摘要：

Effect of a new type of insect chemosterilant, namely, J_{3230} (5-propyl-en- 3-oxy-6-1-methoxyphenyl)ethyl-1,3-benzodioxole), which is a benzyl-1, 3- benzodioxole derivative (BBD) on reproduction of the oriental fruit fly, Dacus dorsalis Hendel, was investigated by feeding larvae or adult insects on the chemosterilant-containing diets. Treatments of larval stage with J_{3230} compound mainly acted on the male adults, causing abnormal spermatogenesis and decreasing egg hatch when mated with untreated females. Examination of spermathecae of these females revealed only few sperms inside and percentage of females capable of accepting from the males treated with 100 ppm was only 3.3%. Histological sections of treated eggs showed homogeneous yolk with cleavage and necrotic egg contents without developing. Newly hatched males and females were fed on a diet containing J_{3230} at 960 ppm from 1 to 9 days after emergence. The subsequent sterility was chiefly due to treating females than males. Electrophoretic analyses of ovarian and egg proteins did not find visible differences in vitellin between treated and untreated females although the treated females slightly delayed their protein deposition.

It seems that J_{3230} is inactive as an anti-juvenile hormone agent for this fly.

Effect of J_{3230} on reproduction of the oriental fruit fly was investigated by exposing matured larvae to top soil treated with this chemosterilant. At concentrations ranging 0.83-3.30mg J_{3230} per g soil, pupation and egg hatch were significantly reduced. Adult emergence was reduced from 77% to 26%, no egg hatch was obtained in the beginning and then restored to the normal gradually. The soil tested was able to retain J_{3230} activity for at least 30 days at concentrations above 1.66 mg/g soil. When soil was subjected to 25°, 30°, and 45℃ for 1 hr, 15 and 30 days respectively, the activity was reduced. When treated for 1 hr, egg hatch could restore to 43.57 and 68%, respectively, and if treated 15 days restored to 59.77 and 85%, respectively. The egg hatch could further restore to 67.85 and 93%, after treated for 30 days. The results showed that activity and persistence of J_{3230} were reduced as rising the treated temperature and prolonging incubation. However the activity was still significantly higher than in the soil treated with acetone alone. When soil was exposed to 254nm UV light for 15hr, or 366nm for 48hr, J_{3230} activity was equally suppressed, revealing that 254nm UV light is more destructive to J_{3230}. When J_{3230} was added to sterilized or nonsterilized soil for 15 and 30 days, respectively, no difference in its activity between two treatments was found. Crossing experiments between treated and control, and males and females revealed that males was primarily affected by J_{3230}. Spermatozoa were also absent in significant number of spermathecae from females which were mated with treated males. Electropherogram of male accessory glands remained unchanged after treating with J_{3230}. Histological sections of testes from J_{3230}-treated males and normal tests all showed normal spermatocytes and spermatids, while sperm bundles were abnormal with aberrant flagella. Therefore J_{3230} compound proved to inhibit microtubule system of male reproductive cells.

RH5992, a nonsteroidal mimics of 20-hydroxyecdysone, could cause larval mortality in all larval stages of the diamondback moth, *Plutella xylostella* (L.), but could not prolong the development. RH5992 also reduced the food consumption by larvae resulting in reduction of pupal weight and adult longevity, and furthermore lowered the fecundity. However, when the eggs were treated with RH5992, percent hatchability was not affected. Additionally, percent pupation and adult emergence were not significantly affected when the larvae were topically treated with RH5992.

Effects of tebufenozide (RH5992) on various stages of the diamondback moth, *P. xylostella* (L.), were investigated by feeding larvae on the kale leaves dipped into different concentrations. This compound was effective to cause larval mortality only when

treated various instars with the concentrations over 400 ppm. Although almost 100% cumulative mortality was obtained by treating 1st through 3rd instars with 400 ppm, the 4th instar was slightly lowered down to 87.5% with 400 ppm but maintained 97.5% with 800 ppm. The LC_{50} values were 96.3, 95.3, 105.8 ppm for 1st, 2nd and 3rd instars, respectively. The LT50 of 4th instar was ranged from 3.4 to 1.7 days at 100 to 800 ppm while it was ranged from 2.3 to 12.3 days for the other instars. The leaf consumption per larva was lower in treatments with 100 ppm or above than the untreated control. The pupal weight, percent emergence, adult longevity and fecundity were affected when newly hatched larvae were continuously reared on the leaves treated with different concentrations of RH5992.

Effects of a juvenile hormone mimic (JHM), pyriproxyfen (code No. S- 31183), on metamorphosis and toxicity to egg, pupae and larvae of the diamondback moth *P. xylostella* (L.), were determined. S-31183 was found to be ovicidal to DBM eggs. The eggs deposited within one day were more susceptible than older ones, and the susceptibility decreased with the increase of the egg age. Histological sections showed that the major effect of S-31183 occurred within 12 hr and was to disrupt embryonic development, resulting in the loss of egg yolk, finally inhibited egg hatch. Application of S-31183 could elicit larval lethality. The LC_{50} value was 6.17, 0.13, 1.04, and 8.48 ppm for larval stages from first to fourth, respectively, when fed with treated rape seedlings . In addition, this compound could inhibit metamorphosis by inducing morphogenetic defects in larvae, pupae and adults. Pupa was found the most susceptible stage to this compound. The LC_{95} for pupae was only 0.02 ppm. Most pupae treated with this JHM did not emerge; few of them which could emerge showed partial eclosion or abnormal wings, causing inability to move, feed and mate.

九、已發表報告目錄：

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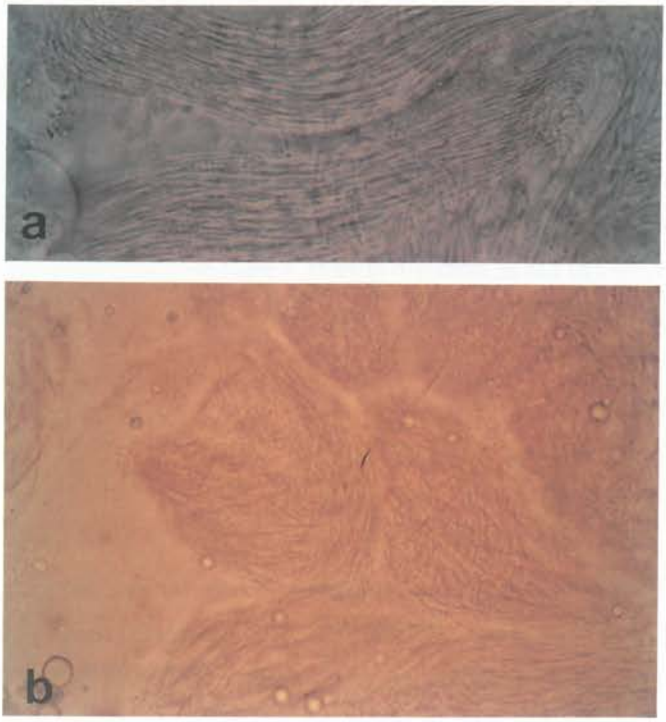
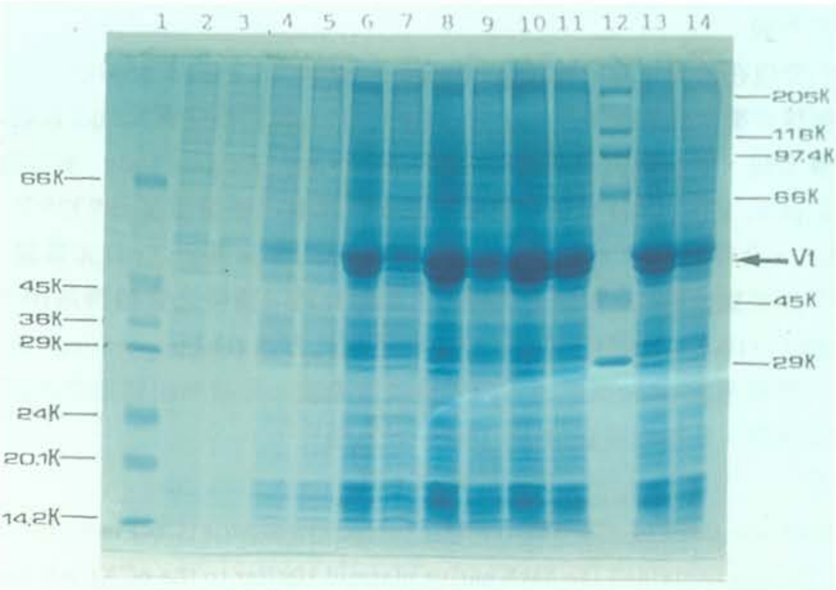
2. Chen, C. C., Y. J. Dong, L. L. Cheng and R. F. Hou. 1996. Deterrent effect of neem seed kernel extract on oviposition of the Oriental fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 89:462-466.
3. Chen, C. C., S. J. Chang, L. L. Cheng and R. F. Hou. 1996. Deterrent effect of the chinaberry fruit extract on oviposition of the diamondback moth, *Plutella xylostella* L. J. Appl. Entomol. 120:165-169.

4. Dong. Y. J., E. B. Yang. L. L. Cheng. C. C. Chen and R. F. Hou. 1997. Effect of topsoil treated with J_{3230} (5-propyl-1-en-3-oxy-6[1-(4-methyoxyphenyl) ethyl]-1,3-benzodioxole) on reporoduction of the Oriental fruit fly (Diptera: Tephritidae) J. Econ. Entomol. 90:535-539.



圖一、東方果實蠅經 Biorational compound, J_{3230}處理後，引起雄蟲精子異常現象。

Fig. 1. Phase contrast photomicrographs of spermatozoa in testes of *Dacus dorsalis* treated Nith acetone(a) or J_{3230} biorational compound(b).



圖二、東方果實驗蠅雌蟲經J_{3230} compound 處理後卵黃蛋白(Vitellin)減少之電泳圖。

Fig. 2. SOS-PAGE profile showing decrease in vitellin after treatment with J_{3230} compound.

一、計畫名稱

中文：利用導管侷限性*Clavibacter xyli* subsp. *cynodontis*構築植物系統性遺傳特性轉殖系統

英文：Construction of an endophytic genetic delivery systen from xylem- limited *Clavibacter xyli* subsp. *cynodontis*

二、執行期限：1993年7月1日至1996年6月30日，共三年

三、執行單位：國立台灣大學植病系

四、美方合作單位及人員：

Rutgers University

Dr. Tseh-An Chen

五、中方研究人員：林長平

六、計畫目的：

本研究乃研擬將非病原性之endophytes-*Clavibacter xyli* subsp. *cynodontis*(Cxc)配合質體載體之選殖而構築成一能轉殖外來基因如proteinaceous pesticides (proteinase inhibitor或Bt toxin 等)至包括turf grasses、玉米、甘蔗等植物之傳送系統，具體目的如下：

(1) 將構築完成一能在Cxc表現之選殖用質體載體 (plasmid vector)。

(2) 將研擬出將外來基因選殖於此一載體並送入Cxc表現之方法。

(3) 將提供此一以遺傳工程改造 (genetically engineered)之Cxc大量送入對象植物之方法，藉以有利往後在植物中表現外來抗病或防蟲之基因。

七、執行成果摘要

我們發現在台灣分離所得之*Clavibacter xyli* subsp. *cynodontis*(Cxc)中，約有三分之一之菌株皆攜帶有質體，且大小多為51kb左右。我們已完成此51kb質體之內限制酵素之酵解圖譜，並發現與陳澤安博士 (Dr. T.A. Chen, Rutgers Univ., 美方合作實驗室)所獲得之pCxc 100之圖譜相似。該質體之複製起點已被選殖至pBR325中，該嵌入片段約4.8kb。合作研究中，由陳博士提供含Bt endotoxin基因之p4850質體，我們其複製起點予以更換成由Cxc質體上選殖出之起點片段，獲得之質體稱為1B-T1，於美方亦獲得1B-1及IB-2。1B-T1、IB-1、IB-2均經電孔方法 (electroporation) 送入Cxc 細菌細胞中，經利用Bt toxin抗體進行西方免疫漬染法，可檢測出轉形之Cxc細胞蛋白中多一條反應帶。此種表現在IB-1、IB-2中較為穩定。

八、英文摘要

About one third of *Clavibacter xyli* subsp. *cynodontis* (Cxc) isolated from various parts of Taiwan contained the 51kb native plasmid similar to the pCXC100 isolated in Dr. T.A. Chen's laboratory (Rutgers University). The restriction maps of the 51kb native plasmid isolated from Cxc on Taiwan had been completed. The restriction map was

similar to that of pCXC100. The origin of replication was identified by cloning various restriction fragments of the native Cxc plasmid into pBR325. A fragment cloned in pBR 325 survived in Cxc indicating that it contained the replicatinng origin (about 4.8 kb containing the origin and it's downstream sequences). A plasmid, denoted p4850, contained the endotoxin gene from Bacillus thuringiensis was provided by Dr. T. A. Chen. We replaced the 3' end of the fragment that contained the origin from Cxc plasmid with the toxin gene fragment (2.8kb), and the plasmid obtained was IB-T1. The plasmid with similar construction obtained from Dr. Chen were IB-1 and IB-2. An elctroporation transfer system for Cxc developed in Dr. Chen's laboratory was followed for transforming Cxc with constructed plasmids IB-1, IB-2, and IB-T1. Using electrophoretic analyses of cellular protein patterns, extra bands of proteins were revealed in Cxc containing IB-1, -2, and -T1, and were recongnized by the antibodies to Bt protein in western blotting analyses. Expressions of IB-1, and IB-2 remained stable after subculturing, wherea that of IB-T1 declined sequentially during subculturing.

九、已發表報告目錄

manuscript in preparation

一、計畫名稱：

中文：木瓜輪點病毒基因在轉型木瓜之表現

英文：Expression of papaya ringspot virus genes in transgenic papaya

二、執行期限：1989年7月1日至1993年6月30日

三、執行單位： 國立中興大學植物病理學系

四、美方單位及人員：

1. New York State Agricultural Experiment Station, Cornell University, New York
   Dr. Dennis Gonsalves

2. University of Hawaii at Manoa, Honolulu, Hawaii
   Dr. Richard Manshardt

五、中方研究人員：葉錫東

六、計畫目的：

選殖臺灣木瓜輪點病毒之鞘蛋白基因，解出其核甘酸序列及轉譯架構，並加以構築使其可在植物細胞內表現，再利用農桿菌之Ti載體系統將病毒鞘蛋白基因轉殖於木瓜細胞內，經由組織培養技術將轉型細胞再分化成為一木瓜植物，此能表現木瓜病毒基因之轉基因植物對該病毒會具有抗性，且其抗性可遺傳至子代成為抗病新品種，對防治木瓜輪點病毒有很大的應用價值。

七、執行成果摘要：

木瓜輪點病毒 (PRSV) 在熱帶及亞熱帶地區為生產木瓜之最主要限制因子。本研究之目的在構築一可在木瓜細胞內表現的木瓜輪點病毒鞘蛋白(CP) 基因，並發展出木瓜組織培養再生技術，從而建立農桿菌媒介將所構築之 CP 基因轉移至轉型木瓜細胞使其再生之模式，本研究植基於由台灣之病毒株系以便獲得台灣本地抗性良好之轉基因木瓜。

第一年之計畫完成了台灣PRSV YK株系的CP基因選殖及解序工作，此提供了構築CP基因於Ti載體的根本基礎。第二年之重點完成了由木瓜葉柄有效生成木瓜植株之組培技術，利用此方法，細菌的GUS基因成功地利用Ti載體由葉柄材料轉移至木瓜細胞。 此外，PRSV CP基因亦被轉入穿梭載體內，而使其能於Ti二元載體內具有轉錄及轉譯功能，此構築好之基因證實可在轉基因煙草中正確表現。第三年時，GUS轉基因木瓜植株順利獲得，其基因之表現並獲酵素聯鎖反應及螢光分析證實，但大部份之GUS 轉基因木瓜之外表形態異常，此可能係再生過程太過漫長 (18 - 20月) 所致。在溫室條件下，PRSV CP之轉基因煙草對其他potyviruses如PeMV，PVY及TEV亦有抗性。PRSV YK RNA之全長序列解序完成後，以病毒本身之5'引導序列所構築之CP基因亦順利完成。至此，我們即有將PRSV 基因體的任一部份以Ti載體轉移至木

瓜細胞之能力。

第四年中，PRSV CP轉基因芽體可由葉柄之轉型細胞再生出來，但效率很低且其外觀畸型不正常，雖然西方漬染證實CP基因可正確表現出來，但正常之植株始終無法獲得。為改善轉型再生之效率，發展出以幼胚分化所得之擬胚組織為培植體，在液體中以金剛砂處理，再以農桿菌感染之方法，此方法證實迅速有效，可取代以葉柄為培植體之方法。

總結本研究之主要成果，即是構築完成可在木瓜表現之PRSV CP基因於Ti二位元載體，並發展出幼胚迅速分化再生之組培技術，從而建立有效可行之農桿菌轉型木瓜之方法，具體完成了本四年計畫 (1989-1993) 所設定的目標，並對日後順利產生具有抗性之CP基因木瓜正常植株提供了堅實之基礎。

八、英文摘要：

Papaya ringspot virus (PRSV) is the major limiting factor for growing papaya in tropical and subtropical areas. This study focused on the construction of a capsid protein (CP) gene of PRSV expressionable in papaya cells, the development of tissue culture systems for regeneration of papaya, and the development of an efficient *Agrobacterium*-mediated transformation system for the CP gene expression in transgenic papaya cells that may regenerate into plants resistant to PRSV infection. The local severe PRSV strain of Taiwan was used as the target virus in this study in order to ensure that protection is specifically against the prevalent strain.

In the first year of project implementation (1989 - 1990), the complete CP sequence of PRSV YK, which is a typical mosaic type strain prevalent in Taiwan, was cloned and determined. The cDNA clone of PRSV YK provided a good base for the construction of the virus CP gene in the Ti binary vector. In the second year, an efficient papaya regeneration system was established by using petiole explants of multishoots derived from mature zygotic embryos. Bacterial GUS gene was first successfully introduced into papaya cells by the Ti- binary vector. In addition, the shuttle vector pGGN-15 containing the GUS leader and a *Nco* I site was generated by site-specific mutagenesis. A *Nco* I site was also created at the N-terminal region of PRSV YK CP gene. By replacement insertion, PRSV YK CP was constructed in the Ti-binary vector in a tarnscriptional and translational manner. Transgenic tobacco plants expressing the constructed CP gene were produced. The results indicated that the construction of the CP gene is correct and can be efficiently expressed in plant cells.

In the third year, some of the GUS gene transfomed calli were regenerated into transgenic plants. The presence of the GUS gene was confirmed by PCR detection and

its expression was detected by fluorogenic assay. However, most of the GUS transgenic papaya plants appeared abnormal, possibly due to a long process of regeneration (18-20 months). Under greenhouse conditions, the PRSV CP transgenic tobacco plants showed broad resistance to challenge inoculation with other potyviruses such as PeMV, PVY, and TEV. After the complete PRSV YK RNA was elucidated, the expression of PRSV CP gene using the homologous 5' PRSV leader sequence was demonstrated in the transgenic tobacco plants. The elucidation of the complete genome of PRSV coupled with the established papaya regeneration system and Ti-transformation system enable us to construct and express any portion of the PRSV genome in papaya cells.

In the fourth year, the CP-transgenic papaya shoots with abnormal appearance were regenerated from transformed cells of *Agrobacterium*-treated petioles with low frequency. The expression of CP gene was confirmed by Western blotting, but normal-looking transgenic plants were not obtained. In order to improve the transformation system, embryogenic tissues derived from premature embryos were first treated with carborundum in liquid phase and followed by *Agrobacterium*-mediated transformation. The novel system proved to be highly efficient and was used in all later experiments, replacing the conventional papaya transformation with petiole as explant.

The construction of the PRSV YK CP gene expressionable in papaya cells in a Ti- binary system, the development of regeneration systems from explants derived from premature embryos, and the establishment of efficient transformation mediated by *Agrobacterim* fulfilled the set goals of this four-year project (1989-1993) and provided a solid base for later work aiming at generation of CP transgenic papaya lines with resistance to PRSV.

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2. Yeh, S. D. 1994. Comparison of the genetic organization of papaya ringspot virus with other potyviruses. Plant Pathology Bulletin 3:54-64.
3. Wang, C. H., and Yeh, S. D. 1994. Comparison of the nuclear iclusion b protein and coat protein genes of five papaya ringspot virus strains distinct in geographic origin and pathogenicity. Phytopathology 84:1205-1210.
4. Yang, J. S., Yu, T. A., Cheng, Y. H., and Yeh, S. D. 1996. Transgenic papaya plants from *Agrobacterium*-mediated transformation of petioles of *in vitro* propagated multishoots.

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一、計畫名稱：

中文：臺灣薊馬傳播番茄斑萎病毒群之研究

英文：Study on thrips-borne tospoviruses in Taiwan

二、執行期限：1994 年 7月 1 日 至 1996 年 6 月 30日

(全程計畫 1994 年 7 月 1日 至 1999 年 6 月 30 日)

三、執行單位：國立興大學植物病理學系

臺灣省農業試驗所植病系

臺中區農業改良場

屏東技術學院

四、美方單位及人員：

Florist and Nursery Crops Laboratory, ARS- USDA, Beltsville, Maryland, U.S.A.

Dr. Hei-Ti Hsu, Dr. Roger Lawson

五、中方研究人員：葉錫東、邱人璋、張清安、陳慶忠、陳滄海

六、計畫目的：

分離並鑑定在台灣感染葫蘆科、豆科、茄科及花卉作物之薊馬傳播番茄斑萎病毒群，針對特定之病毒分離株製備單元及多元抗體以澄清其和番茄斑萎病毒群之血清關係，建立全台灣具代表性 tospovirus 分離株之cDNA 基因庫，選擇對映結構性核鞘蛋白之 cDNA clones (Nucleocapsid, N gene) ，解讀其核酸序列，並加以對比分析，以澄清其分類地位。另外並建立病毒快速診斷及偵測用血清及核酸探針方法，供調查病毒天然寄主範圍、帶毒薊馬之棲群動態及病毒發生生態之用。就特定之病毒分離株研究其薊馬傳播性狀，與媒介昆蟲之專一性及探討病毒於蟲體內複製情形。由上述病毒特性、發生生態及檢定方法之全盤掌握將可提供擬定有效防治策略之參考，以減緩此群病毒對本省作物所造成之經濟損失。

七、執行成果摘要：

在屏東地區調查之結果，發現甜椒受到 TSWV (tomato spotted wilt virus) 及 WSMV (watermelon silver mottle virus) 之感染，而以 TSWV 較為嚴重。 同樣在番茄田調查之結果，所抽取之樣本中受 TSWV 感染者達 67 %，受 WSMV 感染者達 7 %。 自甜椒與番茄田所採集之雜草經 ELISA 測定結果，計有 7 科 22 種植物受番茄斑萎病毒群 (tospovirus) 病毒之感染，菊科與茄科植物之採樣中，攜帶此類病毒之比例尤高。

自下列植物分離獲得 tospovirus 分離株各一株：洋桔梗 (lisianthus), 大岩桐 (gloxinia), 落花生 (peanut) 及 蝴蝶蘭 (phalaenosis orchid)。 經鑑定結果，感染洋桔梗與大岩桐二者， 均屬 TSWV，而感染落花生者則與TSWV、 INSV (impatiens necrotic spot virus) 及 WSMV 皆無血清學關係。自蝴蝶蘭分離之病毒，亦係一種在台灣首次發現

之新 tospovirus 病毒，其血清學性質與 TSWV、 INSV 、WSMV 及自落花生分離之落花生黃化扇斑病毒 (peanut chlorotic fan-spot virus, PCFSV)有別。

中興大學植物病毒研究室及農試所植病研究室為進行番茄斑萎病毒群 病毒的研究，並為供應田間病毒病害診斷之需求，以不同材料為免疫元，已製備完成對 WSMV、TSWV 之不同分離株，以及 WSMV、 INSV 與TSWV 各分離株， 具有專一性之抗血清。連同自國外引進之抗 GRSV (groundnut ringspot virus) 與 TCSV (tomato chlorotic spot virus) 血清及其免疫元，本省植物病毒研究室現已擁有番茄斑萎病毒群之血清型 serogroups I、II、III、 VI 與 V 之病毒及其抗血清。對國內、外未來有關番茄斑萎病毒群之診斷及其他方面研究，當有莫大助益。

在 WSMV 之薊馬媒介傳播方面，發現南黃薊馬(*Thrips palmi*)第一齡與第二齡幼虫，皆有獲毒能力。 薊馬之幼虫或成虫如於其幼虫期獲得病毒， 均可將病毒傳於寄主植物 。將攜帶 WSMV 之薊馬磨出液，以機械方式接種於 *Chenopodium quinoa*，則於接種葉上產生壞疽斑。

在 WSMV 的分生研究方面，承繼本計畫實施前之既有基礎，其 S RNA 及 M RNA 已全部解序完成，計分別有 3545 及 4880 個核甘酸， 二者均為雙極性之構造。 針對其 NSs, NSm, G1-G2 及 N 基因之核酸引子已設計出，可用於反轉錄酵素連鎖反應 (RT-PVR) 檢測之用。 另外針對落花生及蝴蝶蘭之 S RNA 之 cDNA 基因庫亦已構築中，其 N 基因解序後， 二者之新種分類地位即可確立。

八、英文摘要：

A general survey of the sweet pepper fields in the Pingtung area in southern Taiwan revealed the occurrence of both TSWV and WSMV, with the former being more prevalent. A similar survey was conducted for tomato fields. TSWV was again present in 67 % and WSMV in 7 % of the samples examined. Also from the tomato and sweet pepper fields, weeds were collected and subjected to ELISA tests. Results indicated tospovirus infection of 22 different weed species in 7 families. A high proportion of the plant samples in the Compositae and Solanaceae was found to carry tospoviruses.

Tospoviruses were obtained as cultures from the following plants: lisianthus, gloxinia, peanut and phalaenosis orchid. While those infecting lisianthus and gloxinia were identified as isolates of TSWV, the virus infecting peanut is distinct and has no serological relations with TSWV, INSV and WSMV. The tospovirus isolated from phalaenosis orchid also proved to be serologically unrelated to TSWV, INSV, WSMV and the peanut virus, now designated as peanut chlorotic fan-spot virus.

To facilitate serological studies on tospoviruses and for field diagnostic work, antisera were successfully prepared at the Plant Virology Laboratory, NCHU and other

laboratories in Taiwan against the following types of antigen: WSMV, TSWV isolates and the N protein of WSMV, INSV, and TSWV isolates. With the introduction of GRSV and TCSV as well as their antisera, these laboratories are now in possession of tospovirus serogroups I, II, III, VI and V as well as the antisera specific for them. They will be made available for diagnostic studies on tospoviruses here in Taiwan and elsewhere.

In transmission study of WSMV, the first and second instar larvae of *Thrips palmi* proved to be capable of acquiring virus. Thrips at the larval stage as well as the adults that had acquired virus as larva were able to transmit WSMV. Extracts of such adult thrips incited necrotic spots typical of WSMV infection on *Chenopodium quinoa* when mechanically inoculated.

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一計畫名稱：

中文：觀賞植物病毒快速檢測及其防治

英文：Rapid method for diagnosing ornamental plant viruses and their control measures

二、執行期限：1989年7月1日 至 1992年6月30日

三、執行單位：台灣省農業試驗所、國立中興大學

四、美方合作單位及人員：

Dept. of Plant Pathology, Univ of Florida

F. W. Zettler

Floral and Nursery Plant Research, USDA, Maryland

H. T. Hsu

五、中方研究人員：張清安、柯南靖

六、計畫目的：

1. 針對具經濟重要性之觀賞植物所發生之病毒種類進行調查鑑定並確定其對各作物之影響程度。
2. 就已知之重要病毒種類進行偵測試劑之製作與檢定法之發展。
3. 就重要之觀賞植物病毒病害擬定防治對策並加以測試其可行性。

七、執行成果摘要：

在台灣蘭花上曾經被發現之病毒共有五種，其中以齒舌蘭輪斑病毒(ORSV)及東亞蘭嵌紋病毒(CyMV)被視為分布最普遍，對蘭花產業影響最嚴重之二種病毒。本計畫(觀賞植物病毒快速檢測及其防治)之執行乃由1989至1992共四年之期間，其主要目標為針對上述二種病毒進行純化，抗體製作及發展免疫檢查方法以解決蘭花產業受病毒危害問題。本計畫於執行期限內已順利完成ORSV及CyMV二病毒之純化，所使用之方法為參考筆者過去純化菜豆黃化嵌紋病毒(BYMV)所使用之方法為主要架構稍加修改而成。純化ORSV所用之材料乃接種7-10天之 *Nicotiana benthamiana* 葉片。病毒之初步抽取乃使用含0.25% Na_2SO_3之0.1 M potassium phosphate buffer (pH 7.5)緩衝液。植物綠色成份之去除乃使用等量體積混合之chloroform 及carbon tetrachloride 處理。病毒之濃縮與最終之純化則分別利用二次PEG 之沈澱處理與一次Cs_2SO_4等密度離心予以達成。至於CyMV之純化材料則取至接種14天之蝴蝶蘭葉片，利用0.5 M potassium phosphate buffer (pH 7.5) 內含 1% mercaptoethanol 及 0.01M Na_2EDTA 進行抽取，其餘純化之步驟均與上述純化ORSV者完全相同。純化所得試料經電泳分析結果發現二種病毒均只出現單一蛋白帶(protein band)，顯示已不具植物成份污染。另外本試驗亦以電泳分離方式將 ORSV及CyMV 鞘蛋白基本單位(subunit protein)分別加以純化。純化之病毒顆粒及其鞘蛋白經免疫注射於家兔體內後，已成功製備出四種專一性抗血清。以病毒顆粒或鞘蛋白所製備之抗體在雙向免疫擴散反應(SDS-

immunodiffusion test)中均可與其抗原產生專一性沈澱帶，且二種抗體間並無任何明顯差異。此四種抗體均不與健全蘭花及煙草產生反應。除雙向免疫擴散反應外本試驗亦成功發展出更具敏感性與效率性之ELISA及免疫點漬法(Immuno-blotting test)，供蘭花病毒檢測之用。試驗期間所檢測之上千種不同來源之感病株均可順利與上述抗體反應，顯示所製備之抗體與發展之檢測流程已深具實用性。尤其所發展之免疫點漬法可由農友自行採樣並將其印漬於硝化纖維紙(nitrocellulose paper)上，再寄回專業實驗室進行免疫檢測。如此可省去研究人員於現場來回奔波時間，顯著提升病毒檢定效率。本試驗成果已由農林廳自民國82年起開始推廣，於全省各改良場所設置病毒檢定站免費為蘭花業者服務，截至目前保守估計受檢植株已達三萬株以上。

八、英文摘要：

Two viruses, i.e. cymbidium mosaic virus (CyMV) and odontoglossum ringspot virus (ORSV), were recognized as the most important among the five different viral agents which have been found in the orchids of Taiwan. In this project which conducted in the period of 1989 to 1992, the priority was to purify both CyMV and ORSV and to prepare specific antisera againt each virus. The ultimate goal for the project was to develop efficient and sensitive idexing techniques for the diagnosis of these two viral agents in orchids. Eventually both viruses were purified by a procedure similar to the one described for the purification of bean yellow mosaic virus with some minor modifications. ORSV was propagated in *Nicotiana benthamiana* for 7-10 days before harvesting for purification. The virus was extracted from the infected leaves by homogenization in a blender with addition of 0.1 M potassium phosphate buffer, pH 7.5, containing 0.25% Na_2SO_3. Green pigment of leaf extracts was removed by the aids of a 1:1 mixture of chloroform and carbon tetrachloride. After extraction, the virus was concentrated by two cycle of polyethelene glycol (MW 6,000) precipitation and further purified by an equilibrium centrifugation in Cs_2SO_4. CyMV was propagated in Phalennopsis orchids. Infected leaves collected 14 days post-inoculation was homogenized in 0.5 M potassium phosphate buffer, pH 7.5, containing 1% mercaptoethanol and 0.01 M Na_2EDTA. After clarification with a 1:1 mixture of chloroform and carbon tetrachloride, the virus was purified by a procedure similar to that described above for the purification of ORSV. The purities of virus after purification were evaluated and confirmed by SDS-PAGE that only those bands corresponding to capsid proteins (CP) of ORSV and CyMV, but no other protein bands, were detected in the purified samples. The CPs of ORSV and CyMV were also purified by a preparatory SDS-PAGE procedure as previously described. Polyclonal antisera against intact virus particles and purified CPs of ORSV and CyMV were all

prepared in rabbits. All four antisera reacted strongly with their homologous antigens and no differences in the serological reactivities were noticed between antisera against intact virus particles and those against CP in SDS- immunofiffusion tests. None of the antisera reacted with healthy control antigens including orchids and *N. benthamiana* in the same tests. Two other idexing methods including enzyme linked immuno-sorbent assay (ELISA) and immuno-blotting method (IB) which were more efficient and sensitive than SDS- immunodiffusion test were also developed subsequently. Very low background was noticed with the two antisera against virions of ORSV and CyMV when they were used in the ELISA tests. More than thousands of different isolates of ORSV and CyMV from various locaties or diffferent orchid species were tested in the ELISA tests and none of them escaped from the detection by these two antisera, indicating that it is feasible to use these two antisera as sero-probes to index ORSV and CyMV in large-scaled surveys. Another idexing method, IB, was developed mainly for improving the efficiency of sample collection from the fields. Orchid gardeners could proceed the sample collection and even the first stage of virus idexing by themselves. The method involved sampling in fields by making two cuts from the edge of orchid leaves so that a V-shaped leaf disc was collected. Each of the cut surfaces of the leaf disc was directly blotted onto two separate nitrocellulose (NC) paper for immuno-processing by ORSV or CyMV antiserum. The gardener can collect their own samples for indexing and print the cut surfaces of the sample on NC paper, which could be processed in their own laboratory or later sent to a central laboratory for processing without any detrimental effect on the final indexing results. No differences on the indexing results were noticed among pieces of NC paper blotted 2, 4, 8, 16 and 30 days before immuno-processing. The advantages of this method is to allow more efficient field surveys which can be done by orchid gardeners instead of by researchers. All the NC papers with sampled blots can be subsequently sent in to a laboratory and processed by a unified processing protocol. Therefore, the results obtained will be more objective rather than affected by different workers or by different processing environment. Comparing with ELISA test, the sensitivity of IB is equally high. Hundreds of orchid samples have been blindly indexed and identical results were consistently obtained by either of the methods.

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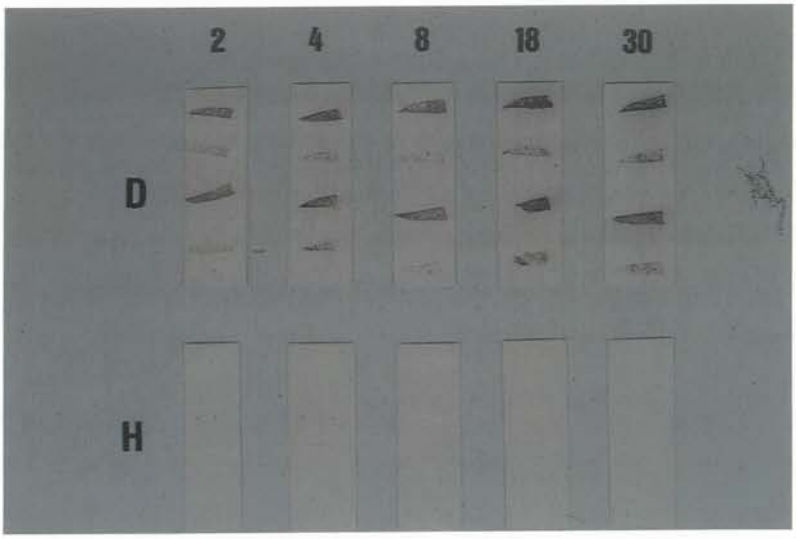
圖一、齒舌蘭輪斑病毒(ORSV)及東亞蘭嵌紋病毒(CyMV)複合感染蝴蝶蘭於葉片上所造成之病徵

Fig. 1. Foliar symptom of phalaenopsis orchid developed by mixed infection of ORSV and CyMV.



圖二、應用免疫點漬法偵測蘭花病毒之結果。圖片上方數字乃待檢樣品點漬於硝化纖維紙後置於室溫下儲藏至進行免疫反應之天數，結果顯示點漬後樣品儲藏一個月再進行免疫反應仍不會影響結果之正確性。

Fig. 2. Results of immuno-blotting test to detect orchid viruses. The numbers on the top indicates the period (days) after blotting samples on NC paper before immunoprocessing. No differences are found on the intensities of color reaction among different storage periods before immuno-processing.

一、計畫名稱：

中文：利用遺傳工程誘導對蘭花喜姆比蘭嵌紋病毒產生抗性之研究

英文：Genetically engineered resistance to cymbidium mosaic virus in orchid

二、執行期限：1993年7月1日至1997年6月30日，共4年。

三、執行單位：鳳山熱帶園藝試驗分所

四、美方合作單位及人員：美國農業部 Beltsville center Dr Hei-ti Hsu

五、中方研究人員：王惠亮

六、計畫目的：

台灣花卉產業近三年來急速成長，栽培面積六千餘公頃，年產值估計有新台幣二十億元，其中又以高價位之蘭花為重要之花種，初期以盆栽為主，近年來以切花為大宗。蘭花繁殖除以雜交之種子繁殖外，尚可用無性分株法繁殖，分株通常用刀剪分切，此分株法和以刀採收新鮮切花時，為病毒病傳播之主要途徑。謝氏和韓氏(1975) 曾報告，某些栽培園患病毒病竟高達一半以上，而以嘉德麗亞最為普遍，此外蝴蝶蘭、石斛蘭、萬代蘭、文心蘭、喜姆比蘭皆有發生，而此毒素病大部分皆由喜姆比蘭嵌紋病毒(Cymbidium mosaic virus) 所引起，張氏和李氏 (1990) 報告為害本省蘭花之病毒主要為喜姆比蘭嵌紋病毒 (CyMV) 和Odontoglossum ringspot virus(ORSV)。CyMV較ORSV為嚴重。蘭花罹患病毒病後，影響蘭花之壽命及花之品質、花色、外觀甚巨。目前蘭花之病毒病，除了罹病株拔除燒毀外，只利用熱或化學藥品，如 5% NaOH 來消毒蘭園工具，防止人為機械傳播，尚無較有效積極之防治。目前工作著重於病毒之偵測，張氏製作之多株抗體供全省蘭花病毒診斷服務站使用。近年來生物技術在農業上之應用頗有進展，利用病毒鞘蛋白基因之遺傳工程交護保護法最早由Powell- Abel以TWV鞘蛋白基因為例子提出報告。接著其他許多病毒之利用鞘蛋白基防治研究均獲得相似的結果。Hiatt於1989首先報告利用轉殖植物生產抗體。此研究結果亦顯現一個啟示，即是植物病毒病害之防治可能可以藉由具抗體基因之轉殖植物來達到目的。本研究之目的即在於經由具有CyMV之鞘蛋白基因或抗體基因之轉形植物來達到有效防治CyMV病害。

七、執行成果摘要：

來自美國農部獲得之CyMV之五個融合瘤細胞株重新以RPMI培養液於5%二氧化碳，37℃培養箱中培養，其中二個細胞株正常生長產生之抗體經過ELISA測定，均對CyMV有很強之反應。經Isotyping測定均屬於IgG3 重鍵與Kappa輕鍵。利用Acid quanidium-phenoi-chloroform方法及。oligo (dT) chromatography方法，獲得高純度及高產量之mRNA。以mRNA當作模板，利用反轉錄酵素及primer(Oligo (dT) *Xho* I)進行cDNA之第一股之合成，再以RNaseH和DNA polymerase I合成第二股cDNA，建立cDNA Liberary。另外利用polyethylene glycol沈降和離心法，獲得高純度及高產重之

CyMV，再經由 phenol和phenol chloroform方法萃取病毒之RNA。再利用Viral RNA當模板，以oligo (dT)當primer，而獲得3'end之coat protein gene之cDNA。獲得之cDNA經過南方墨點分析測試，以CyMV探針偵測，可得到明顯反應。利用雙去核甘酸解序法進行解序，長度為800bp，CP基因利用pBIN 質體已完成結合並利用基因鎗轉殖進入蘭花之泥胚，目前有1180株幼苗於NPT II ELISA檢定，呈正反應，取其中10株強反應之幼株，有5株於PCR 反應中，顯示基因之產物，抗體基因之重鍵及輕鍵cDNA 已完成選殖及核酸序列定序。

八、英文摘要：

Cymbidium mesaic virus are the problem of most conern to orchid growers in Taiwan. The purpoes of this research is control CyMV througth the transgenic orchid epressing either CyMV coat protien or antibody to CyMV. 800bp of coat protien gene of CyMV was cloned and constructed into pBIN expressing vector More than ten thousand orchid embryos were transformed with CP gene by using particle gun 10％ of embryos were survival in karamycin selection medium 1180 seedlings of these survived orchid in the medium were identified with positive NPT II reactions. Southern, northern, western, and challange analysis were investigating. Five of ten seedlings wish NPT II positive were shown geve product by using PCR tchnigur. Antibody gene of a hybridoma cell line provided by Dr. H. T. Hsu in USDA was also cloned 1.6 kb of the heavy chain of antibody gene was sequenced and identified to be a full length fragement belonging to lgG 3 subclass 0.9 kb of full lenth of a kappa light chain was also sequenced.

一、計畫名稱：

中文：台灣重要作物 Closterovirus 病害之診斷與防治技術研究

英文：Studies on the diagnostic and control techniques of closterovirus diseases among important crops in Taiwan

二、執行期限：1993年7月1日 至1997年6月30日

三、執行單位：國立中興大學植病系

四、美方合作單位及人員：

1. Center for Engineering Plants for Resistance Against Pathogens, U.C. Davis
George Bruening

2. USDA-ARS, Department of Plant Pathology, U.C. Davis
Deborah Golino

3. Foundation Plant Material Service, U.C. Davis, Adib Rawhani

五、中方研究人員：曾德賜、陳慧璘、唐益國、劉逸琪

六、計畫目的：

本研究為一四年期中美合作研究計畫，主要目的在建立本省重要作物，包括葡萄、柑桔等當中closterovirus群病毒之快速偵測檢定技術，終極研究目標除以提供我國檢疫與防疫工作之實際應用參考外，並將由而建立無病毒種苗之鑑別、保存、繁殖及栽培應用之完整工作體系，以奠立本類病毒防治工作之基石。

七、執行成果摘要：

本研究主要工作目標在針對柑桔南美立枯病 (Citrus tristeza virus，CTV) 與葡萄捲葉病 (Grapevine leafroll associated virus，GLRaV) 兩台灣地區發生相當普遍之重要Closterovirus病毒病害，建立其病害快速診斷及生物防治有關之生物技術。基於病毒病害防範於未然之優先考量，快速診斷技術之發展乃本計畫主要工作重點。第一、第二年期計畫中，利用本研究所建立之病毒純化改進方法，已順利純化得GLRaV病毒，並成功製備得可提供本病毒免疫偵測應用之多元抗血清及可產生專一性單元抗血清之融合瘤細胞株。此外利用所創用Closterovirus 病毒dsRNA 純化分析改進方法，已進而生合成其對應 cDNA；配合黃秋雄先生提供之CTV抗血清與上述製備之GLRaV抗血清，並已分別以免疫篩選法由CTV與GLRaV 之cDNA Library 篩選獲得具生合成兩對應病毒鞘蛋白能力之*E. coli*選殖株；第三年期計畫中，CTV鞘蛋白基因已解序完成，利用純化自 *E. coli* 選殖菌株培養之CTV鞘蛋白，經免疫注射大白兔所獲 CTV 抗血清，以 ELISA 法檢測 CTV 病毒且已證實其敏感度、專一性均符合免疫偵測之需要。於第四年期計畫中，以對應巨峰葡萄上第三型GLRaV之抗血清篩檢所獲得之GLRaV鞘蛋白基因亦已解序完成，其與CTV鞘蛋白基因之同質性甚低，對應蛋白之序列解

讀尚待繼續努力中。在本類病毒核酸探針偵測技術建立上，由CTV、GLRaV之鞘蛋白基因所製備之cDNA探針在本類病毒RNA偵測上，均已證實可達pg之敏感度，而由其序列所設計之引子對，以RT-PCR法更已證實敏感度可達fg程度，就檢、防疫上病害診斷工作之需要而言，已符合快速、方便、準確等要件。

八、英文摘要：

The main objective of this investigation was to establish a fast and accurate disease diagnostic biotech system for and the biological control of diseases caused by citrus tristeza virus (CTV) and grapevine leafroll associated virus (GLRaV). The two diseases have been shown to be widespread in Taiwan and are one of the main limiting factors for the fruit production. As prevention of the introduction of diseased propagating material is a determinative factor for the control of both diseases, the main emphasis of this investigation was on the development of the fast diagnostic tools and techniques. In the first two years, we have succeeded in purification of GLRaV from diseased grapevines using an improved purification protocol developed. With the purified virus preparation, polyclonal rabbit antiserum as well as serotype III GLRaV specific monoclonal antibodies were developed. The antibodies produced were shown by ELISA to be useful tool for the indexing of GLRaV infection. For cDNA library construction, methodology with improved efficiency of dsRNA purification was established. The cDNA library was later constructed for CTV and GLRaV respectively. With the CTV specific antibody provided by Mr. C. H. Huang of TARI and the GLRaVspecifc antibody we have as screening tool, recombinant *E. coli* clones carrying coat protein (CP) gene of CTV and GLRaV were obtained. In the third year project, sequencing of the obtained CTV CP gene was completed. CTV CP produced from the recombinant *E. coli* clone was purified and used to prepare polyclonal antiserum. The sensitivity and specificity of thus prepared antiserum was demonstrated by ELISA to be satisfactory in regard to the indexing of CTV infection. In the fourth year project, sequencing of GLRaV CP gene was completed with a recombinant clone obtained through immunoscreening using type III GLRaV antiserum developed from diseased Kyoho grapevine. Resolution of the encoded protein sequence is currently underway. For the disease indexing of CTV and GLRaV, northern blotting analysis has demonstrated that sensitivity of the cDNA probes prepared from these CP gene containing fragments were around pg level of the respective complementary viral RNAs. Furthermore, with the virus specific primer pairs constructed from the resolved CP gene sequence, detection of the closteroviral RNAs by RT-PCR was shown down to fg level. For the need of practical disease inspection, the techniques developed in this four

-year-term project have met the requirement of being fast, convenient, and accurate; and the knowhow is ready to spin-off.

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5. Tarng, Y. K. 1994. Molecular cloning of coat protein gene of citrus tristeza virus in Taiwan and its application in virus detection. Master Thesis, Department of plant Pathlogy, National Chung Hsing University.

一、計畫名稱：

中文：利用生物技術轉移色素合成基因創造新花色之研究

英文：Developing new flower colors by transformation of pigment biosynthetic gene(s).

二、執行期限：1994年12月1日至1999年6月30日，共四年六月。

三、執行單位：臺灣省農試所農藝系，種苗改良場技術課，中興大學農藝系。

四、美方合作單位及人員：

Floral and Nursery Plant Research, U. S. National Arboretum

Dr. R. J. Griesbach

USDA, Maryland, USA

Dr. H. T. Hsu

五、中方研究人員：

王強生、夏奇鈮、陳駿季、宋濟民

六、計畫目的：

花色為決定切花作物產業及商品價值之重要因素。許多花卉作物如晚香玉、金花石蒜及蝴蝶蘭等，花型優美、高貴具有高級切花所需優點，但因花色少而無法大眾化。由於可供利用之花色種原有限，因此花色育種亦受限制。本計畫之目的，即將研究晚香玉色素合成途徑，探討其無法產生其他色素之生化及分子障礙，進而利用生物技術突破傳統雜交障礙與基因來源限制，將特定之花色基因轉移至晚香玉創造新的花色，提高其商品價值。

七、執行成果摘要：

建立花色素之萃取方法，以薄層分析(TLC)及高壓液相層析(HPLC)技術，進行色素產物分析，探討晚香玉色素合成途徑之障礙。

進行基因庫蒐集已發表之色素基因序列設計、合成核酸引子，利用聚合酵素連鎖反應(polymerase chain reaction, PCR)技術選殖晚香玉花色基因片段，發現白色晚香玉至少因缺乏一種DFR基因而無法合成色素產物，如能轉移一有效DFR基因將可提供新花色。利用RT-PCR (reverse transcription - PCR) 從黑色大豆(青仁烏豆)種皮我們已選殖到一DFR基因片段，其長度為232 bp。利用此一 DFR片段篩選cDNA基因庫，現已獲得41個殖系其長度為1.2 kb至2.1 kb，目前正進行基因定序工作。

建立高效率的組織培養及再生系統為基因轉殖所必須。本試驗利用晚香玉小花梗為培植體，以含TDZ之培養基於黑暗中培養一週可獲得最高植株再生率(72.9 %)。現正以此再生系統為基礎，建立以基因鎗為主之基因轉殖系統，進行DFR基因轉移，創造新花色。

八、英文摘要：

Flower color has become one of the major goal in the flower crops breeding program. The objective of this project is to develop new flower color(s) by transformation of pigment gene(s) through biotechnology to tuberose which only the white flower, is commercially available. Molecular study on the anthocyanin biosynthetic pathway by RT -PCR showed that the white color tuberose is due mainly to a deficiency of the dihydroflavonol reductase (DFR) gene. Therefore, transformation of a active DFR gene to the white tuberose may provide new flower color(s). A black seedcoat soybean variety Chin-Ren-Woo-Dow (CRWD) was chosen as resources of pigment genes for the flower color(s) engineering in tuberose. Degenerate primers designed by GeneBank search and sequences analysis were used to clone the DFR gene from the black seedcoats of CRWD by RT-PCR. A 232 bp soybean DFR fragment was cloned and applied to screen a cDNA library. So far, 41 cDNAs range from 1.2 to 2.1 kb have been cloned and the sequence analysis is undergoing. Tissue and plant regeneration system is developing to establish a gene transformation system for tuberose. A recent experiment showed that the explants of flower pedicels cultured on medium containing TDZ with one week dark incubation resulting the highest regeneration rate (72.9 %). A particle bombardment transformation system based on this regeneration system for tuberose pigment gene transfer is undergoing.

一、計畫名稱：

中文：天國栗高硫蛋白質基因在毛豆及綠豆的表現

英文：Expression of sulfur-rich protein gene from paradise nut in mungbean and vegetable soybean for nutritional quality improvement

二、執行期限：1993年7月1日至1998年6月30日，共5年。

三、執行單位：亞洲蔬菜研究發展中心作物改處

四、美方合作單位及人員：

Dept. of Plant Mol. Physiol.
U. of Hawaii, USA
Dr. Samuel S. M. Sun

五、中方研究人員：宋達、嚴慧心、柳建安、郭忠吉

六、計畫目的：

建立毛豆及綠豆之基因轉殖系統，俾將天國栗高硫胺基酸蛋白質基因殖入亞蔬中心所育出之高產毛豆(AGS 292與高雄2號)及高產、抗病之綠豆(NM 92與VC 1973A)品種。

七、執行成果摘要：

1.再生及繁殖系統的建立

毛豆子葉培養於含1mg/l BAP，0.7mg/l GA3之MS培養基兩週後可誘導不定芽形成。芽體轉植於含0.1mg/l NAA的MS培養基約二個月後可使其發根。

綠豆子葉培養於MBB5培養基(MS無機鹽+Gamborg維生素+5mg/l BAP) 約10天後可誘發不定芽，約一個月後可移至發根培養基(MS培養基+1g/l KNO_3+0.2mg/l IBA)誘導生根。

2.基因轉殖系統的建立

a.農桿菌感染法

比較不同農桿菌品系與質體組合對綠豆與毛豆之感染力後，選定農桿菌品系A281(pMT1)及LBA4404 (pBI121)分別感染綠豆種子及子葉。經點墨雜交法和PCR偵測顯示26%之VC 1973A種子及1%子葉帶有GUS基因，而NM 92品種則未測得任何植株含有GUS基因。

b.花粉管基因轉殖

以帶有報導基因GUS及標識基因NPT II之質體pBI121經由花粉管基因轉殖方式，篩得6.2%高雄2號及1.7% AGS 292的毛豆植株對kanamycin具有抗性。

c.農桿菌配合氦鎗粒子撞擊法進行基因轉殖

研究結果顯示綠豆VC 1973A子葉以1.0 μm金粒6mg/DNA 100 μg、壓力1350-1550 psi 撞擊效果較佳。撞擊後再以LBA4404 (pBI121)感染，結果測得10.7%之培殖體

具有GUS活性。

研究結果顯示毛豆及綠豆之再生與繁殖已可順利進行。以農桿菌感染或配合氣鎗粒子撞擊進行綠豆之基因轉殖殊為可行。未來將應用於毛豆之基因轉殖，期能獲得轉型植株。

八、英文摘要：

1. Development of regeneration system:

Soybean: Multiple shoots were induced two weeks after mature cotyledons were cultured on MS medium supplemented with 1mg/l BAP and 0.7mg/l GA3. Roots were formed two months after the adventitious shoots were transferred to rooting medium (MS medium with 0.1 mg/l NAA).

Mungbean: multiple shoots were induced from cotyledons after cultured for about ten days on MBB5 medium (MS salts + B5 vitamins + BAP 5mg/l) and were transferred to rooting medium (MS medium + KNO_3 1g/l + 0.2mg/l IBA).

2. Establishment of gene transformation system

a. *Agrobacterium* infection method

After comparing infection ability of different combinations of *Agrobacterium* strains and plasmids, *Agrobacterium* strains A281 (pMT1) and LBA4404 (pBI121) were chosen to infect seeds and cotyledons of mungbean. Results of dot blotting and PCR detection indicated that 26% seeds and 1% cotyledons of VC 1973A contained GUS fragments. However, no GUS fragments were detected in infected seeds or cotyledons of NM 92.

b. Pollen-tube method

Plasmid pBI121 carrying NPT II selective marker and GUS reporter gene were used to transform soybean through pollen tube pathway. Results indicated that 6.2% seeds of Kaohsiung No. 2 and 1.7% seed of AGS 292 were resistant to kanamycin.

c. Microprojectile bombardment in conjunction with *Agrobacterium* infection

Research data suggested a bombarding condition for mungbean cotyledons: ratio of 6mg gold particle (1.0 μm)/100 μg DNA and pressure at 1350-1550psi. Cotyledons of VC 1973A were bombarded and then infected with LBA4404 (pBI121). Ten point seven percent of cotyledons showed GUS blue spots.

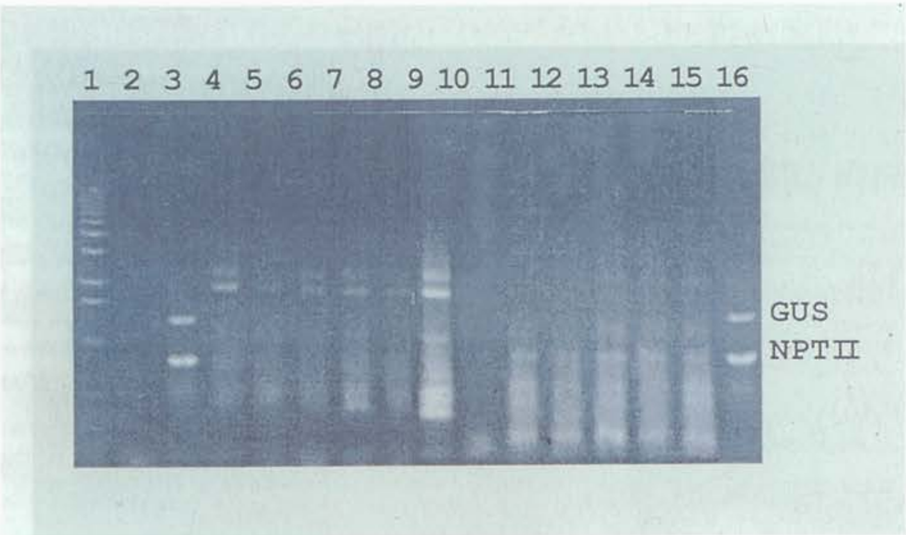
In summary, regeneration systems for mungbeans and vegetable soybeans were established. Research data indicated the possibility of transforming mungbean by *Agrobacterium* infection or in conjunction with microprojectile bombardment. These two methods will be applied on soybean transformation to obtain soybean transgenic plants.

圖一、大豆品種AGS 292 植株之再生。

Fig 1. Plantlet regeneration from a cotyledon of soybean variety AGS 292.

圖二、綠豆品種NM92叢生芽之誘導

Fig 2. Induction of multiple shoots from a cotyledons of mungbean NM 92.



圖三、綠豆品種VC 1973A再生植株轉殖基因PCR之分析。

第1行，核酸分子量標識；第二行，對照組第3-9行，通過抗生素篩選之再生植株之DNA增殖第10-15行，未經農桿菌感染之再生植株之DNA 增殖第16行，增殖之載體PMT1。

Fig 3. PCR analysis of putative transgenic plants regenerated from Agrobacterium -infected cotyledons of VC 1973A. Lane 1, DNA size marker; Lane 2, blank ; Lane 3-9, DNA samples from putative transgenic plants; Lane 10-15, Negative control; Lane 16, plasmid positive control.

中美農業科學合作研究計畫執行結果報告

Final and Progress Reports of COA-USDA Agricultural Science Cooperative Projects

林業領域 (Forestry)

一、計畫名稱：

中文：美國木材在台灣之市場調查

英文：Marketing research of Imported Timber from the United States in Taiwan

二、執行期限：1987 年 7 月 1 日至 1989 年 6 月 30 日

三、執行單位：林業試驗所林業經濟系

四、美方合作單位及人員：

東北森林試驗站 (Northeastern Forest Experiment Station)

之林業科學實驗室(Forestry Sciences Laboratory)

布魯斯・韓森 博士 (Dr. Bruce Hansen)

五、中方研究人員：任憶安

六、計畫目的：

(1) 蒐集並瞭解台灣及美國木材貿易變動情形。

(2) 瞭解木材加工製造工廠對美國木材使用狀況。

(3) 評估台灣木材加工業未來趨勢。

(4) 評估美國木材在台灣市場反應。

七、執行成果摘要：

(1) 調查樣本廠商中，有 83% 表示有用美國木材，17% 表示沒有用美國木材。在有用美國木材的廠商中，有 34% 表示是針葉樹木材及闊葉樹木材都有用。有 24% 表示是只用針葉樹木材，而有42% 表示只用闊葉樹木材。

(2) 在使用闊葉樹木材廠商中各主要樹種使用百分率如次：

Oak(35.2%)，Maple(13.6%)，Ash(12.8%)，Alder(10.4%)，Walnut(8.0%)，Birth(5.6%)，Cherry(4.0%)等。

(3) 在使用針及闊葉樹木材之廠商中，各主要樹種使用百分率如次：

Oak(23.4%)，Maple(8.5%)，Ash(13.5%)，Alder(14.2%)，Walnut(9.2%)，Birth(8.5%)，Cherry(5.7%) 等。

(4) 在使用闊葉樹木材之廠商中，使用三種木材者就占 75%，在使用針及闊葉樹木材之廠商中，使用三種木材者就占 63%，表示台灣廠商使用美國木材樹種較集中於少數樹種。

(5) 台灣廠商對未來美國木材使用多半認為會增加。

八、英文摘要：

On July 1, 1987, a cooperative study of the Taiwanese market for American hardwood and softwood timber was initiated between the Taiwan Forestry Research Institute and the USDA Forest Service, under the auspices of the Office of International Cooperation and Development. Taiwanese users of U.S. timber were surveyed during the

fall of 1988. A total of 143 questionnaires were received in response to this survey. Of these, 24 indicated that they did not use American timber, 50 used only hardwood, 28 only softwood, and 41 both hardwood and softwood.

Generally it was found that respondents tended to restrict their use of American timber to a relative few species, but were less restrictive in thickness and grade. Oak was clearly the predominate hardwood species. Hemlock, red cedar and Port Orford cedar were the most often mentioned softwood species in use. Overwhelmingly, respondents were of the belief that their future use of U.S. timber would increase.

九、已發表報告目錄：

(1) 台灣進口美國木材市場之研究 (林產工業 7(2):47-59(1988))

(2) 美國木材在台灣使用狀況之剖析 (林產工業 9(3):67-76(1990)

一、計畫名稱：

中文：高比重闊葉樹材之加工

英文：Machining and Gluing of High Density Hardwoods

二、執行期間：1987年7月1日至1991年6月30日，共4年。

三、執行單位：台灣省林業試驗所利用系

四、美方合作單位及人員：

Southern Forest Experiment Station,

Dr. C. Y. Hse

五、中方研究人員：唐讓雷、黃彥三、周群、黃國雄。

六、計畫目的：測試高比重闊葉樹材加工性，開發木材料源。

七、執行成果摘要：

取用比重介於0.5至1.0間之闊葉樹材，作為機具切削、膠合、接合設計與切片試驗。重要測試結果說明，切削力與深度之間有顯著正相關，在不同測試樹種間均一致顯示此相關結果，而切削力隨著斜度角之減少而增加，不過，斜度角之增大，對木材切削面之品質漸有不利之效果，試驗結果指出，切削作業必需在小於臨界斜度角之刀具下進行，以避免撕裂木理之發生，臨界斜度角視高比重闊葉材之種類而異。鑽切測試在木材之徑面或弦面上進行，結果說明鑽孔內端之內壁粗糙度，隨鑽切次數之增加而漸增，然而鑽孔之中段及外端之內壁粗糙度，則呈與上述相反之趨勢，鑽切之動能消耗隨切削次數呈指數曲線增加，又動能之消耗依下列狀況而依序遞減：鈍鑽頭（經兩萬次鑽切後）在徑向木理切削＞鈍鑽頭在弦向木理切削＞利（新）鑽頭在弦向木理切削＞利鑽頭在徑向木理切削。鑽孔孔隙之尺寸精度隨鑽頭使用時間之延長而降低，試驗結果亦說明，鈍鑽頭常導致鑽孔孔徑之稍微下降。

高比重闊葉樹材之膠合試驗顯示，木材比重與常態膠合強度之間，並無密切相關，但是較高比重之木材，得到膠合耐久性較差之結果，這可歸因於膠合層面，在較高比重木材之狀況下，需承受較高程度應力集中之抵抗，而導致膠合面衰退。若使用不同樹種之木材相互膠合，則可發現二者之間，較低比重之木材，常控制著膠合剪力強度之結果，同時亦顯出較高之木破百分率。在木材接合之測定中，由靜曲曲線之比例限度斜率與構件比重之間，獲得顯著之正相關，木材接合之設計，受下列測試因子而影響；即膠合劑之種類、接合部位之密合度比率、接合構件之比重及膠合方法等。木材裂解切片測試說明，木片之品質隨木材及軟化處理方法而異，高比重木材常產生木材捲曲之結果，此捲曲程度是謂其後續加工上最具影響之因子。

八、英文摘要：

High density hardwoods with wood specific gravities ranging from 0.5 to 1.0 were used for machining, gluing, joint designing and flaking tests. The test results indicated

that a significantly positive correlation was found between cutting force and depth. This correlation was shown consistently for different wood species. The cutting force increased with the decrease in the rake (cutting) angle. Nevertheless, the increase in the rake angle showed some detrimental effects on the surface quality of the machined wood. It is proved that cutting should be done within the critical rake angle, which varies with wood species, so as to avoid the occurrence of torn wood grains. Boring of wood was conducted on both quarter-sawn and flat-sawn boards. The test results pointed out that the surface roughness of the inner end portion of a bored hole increased with the increase in the number of boring cuts, whereas the surface roughness of the middle and outer end portions decreased. The power consumption of boring machine increased with the time of use and the increase was shown in an accelerating rate by the regression analyses. The test of wood boring also suggested that the power consumption of machining changed in the decreasing order as: dull (after 20,000 times of boring cuts) bit at the radial wood grain direction > dull bit at the tangential direction > sharp (new) bit at the tangential direction > sharp bit at the radial direction. The size accuracy of bored hole dropt as the use time of boring bit prolonged. There was an indication that the bored hole became slightly smaller when a dull bit was used. The glue bond study showed no close correlation between normal bonding strength and wood density. Higher density hardwoods gave lower bond durability, however. This may be attributed to the greater stresses imposted on the gluelines by the higher density wood. Testing of gluing between different wood species indicated that the wood with lower density of the two chosen species governed the glue bond shear strength and gave a much greater percent wood failure. Tests on the wood joint showed that the the proportional slope in bending increased with increasing the density of the wood joint components. The joint designs were affected by adhesive type, fitness ratio, component specific gravity, and glue spreading method. The flaking test indicated that the chip quality varied with the wood softening treatments. The degree of chip curliness may be the major problem affecting its uses for further processing.

、已發表報告目錄：

1. Tang, J. L. and G. S. Hwang. 1990. Gluing properties of high density hardwoods. Proceedings of the Joint International Conference on Processing and Utilization of Low-Grade Hardwoods and International Trade of Forest- Related Hardwoods., Taipei, Taiwan, ROC. p243-251.

2. Tang, J. L. and I. A. Jen. 1992. Current timber consumption situation in Taiwan. Proceedings-An International Perpective of Wood Science & Technology and Issues Affecting Wood Utilization., Madison, WI., USA. p1-7.

3. 周群、唐讓雷 1992. 木釘接合之靜曲與拔出強度(一) 林業試驗所研究報告季刊。7(3)：249-257。

4. 周群、唐讓雷 1993. 木釘接合之靜曲與拔出強度(二) 林業試驗所研究報告季刊。8(1)：61-83。

一、計畫名稱：

中文：利用美國櫟樹小徑木栽培香菇之研究

英文：Cultivation of shiitake with small diameter logs of american oaks

二、執行期限：1986年12月2日至1990年12月1日，共四年。

三、執行單位：台灣省林業試驗所

四、美方合作單位及人員：

Department of Forestry, University of Florida, Gainsville, Florida

Dr. Roger Webb

五、中方研究人員：鍾旭和

六、計畫目的：

To study the potential and feasibility of cultivating shiitake in Taiwan with small diameter logs of American oaks.

七、執行成果摘要：

隨著生活水準的提高，國人對香菇的需求在質與量皆與日具增，但近年來自然資源保育觀念及環保意識的急遽興起，致林業政策以不砍伐天然闊葉林為原則，因此造成本省菇木來源短缺，本研究針對美國大面積櫟樹小徑木為材料進行香菇栽培的可行性研究。惟本期中美研究計畫美方未提撥研究經費，原計畫中主要重點的小徑木栽培香菇試驗項目無法執行，僅能以台灣木材工廠收集到的美國紅櫟樹(red oak)與白櫟樹(white oak)木屑做太空包栽培試驗。試驗處理包括三種木屑(紅櫟木屑、白櫟木屑及本省菇場所使用木屑)、三種香菇菌種(中興領菌種、羅東菌種及金門菌種)以及兩處栽培地(南投蓮華池、宜蘭棲蘭山)，試驗結果顯示：栽培地以棲蘭山優於蓮華池，原因可能是氣候因子所致；三種菌種的產菇量以羅東菌種最高，中興領次之，金門所分離的菌種最差；不同木屑的香菇產量以白櫟樹為最高(每包320g)，紅櫟樹(213g)次之，本省菇木屑(165g)最低，顯然美國櫟樹極適宜在台灣供作香菇栽培之用。若能經適當規畫，利用美國櫟樹小徑木可為國內香菇栽培產業提供另一新契機。

八、英文摘要：

The raise of the standard of living here in Taiwan has increased the demand, both in terms of quantity and quality, of shiitake. However, there is a shortage of supply of domestic logs for shiitake cultivation due to the change of forest policy which has called for a ban on logging of natural forest. Our project, therefore, was to study the potential and feasibility of cultivating shiitake in Taiwan with small diameter logs of American oaks which represent one of the largest sources of under utilized wood in the United States. Since, during this study, funding from USDA which is needed to collect and ship the oak logs from the United States to Taiwan was not available we, therefore, was unable

to carried out the key portion of the experimental design proposed. In stead, we collected sawdust of American red oaks and white oaks from local saw mills and compare their effectiveness as shiitake cultivation substrate with the sawdust commonly used by local shiitake producers. Different sawdust packed in polypropylene bag as a medium was inoculated with three different shiitake fungi, Lentinu edodes (Berk), strains viz. Chung -Hsing-Lin, Lo-Tung and Kin-Men and cultivated at Lien-Hwa-Chih, Nantou and Chi-Lan -Shan, I-Lan. Experimental results showed that shiitake cultivated in Chi-Lan-Shan is more productive than that maintained at Lien-Hwa-Chih indicating that the climate of the former is more suitable than the later for shiitake cultivation. Among the fungi strains tested the Lo-Tung strain is the best followed by Chung-Hsin-Lin strain; and the Kin-Men strain is the least productive one. Results of testing different sawdust as cultivating substrate showed that the white oak which produces 320 grams of shiitake per polypropylene bag is the best medium followed by that of red oak which yields 213 g/bag. The sawdust substrate commonly used by local shiitake grower only produce 165 g/bag. These results suggest that there is a great potential to use American oaks, logs or sawdust, for shiitake cultivation in Taiwan. With careful planning there is a great opportunity for Taiwan to re- vitalize its shiitake production industry by using the low grade and small diameter logs of American oaks.

九、已發表報告目錄：(無)

一、計畫名稱：

中文：菌根接種與採礦棄土石地復舊造林

英文：Mycorrhizae and establishment of trees on mine spoils

二、執行期限：1986年12月2日至1990年12月1日，共四年。

三、執行單位：台灣省林業試驗所

四、美方合作單位及人員：

Institute of Mycorrhizal R & D, Southeastern Forest Exp. Station, Forestry Sciences Lab.

Dr. Donald H. Marx

五、中方研究人員：鍾旭和

六、計畫目的：

To investigate the mycorrhizal status of plants occuring in mine spoils and in natural environment; To study the ecological adaption of mycorrhizal fungi in mine spoils and the effects which affect mycorrhizal association; and To asses the possible role of mycorrhizae in establishing trees on mine spoils.

七、執行成果摘要：

人類對礦產的開採會對當地的水土保持產生徹底的破壞，尤其在本省地形陡峭雨量集中的特性下，採礦棄土地對土壤流失、水質惡化更是嚴重，加上廢土石地土壤貧瘠保水不易，因此復舊造林極為困難。本研究針對本省煤礦與石灰石礦兩種棄土石地，進行復舊造林試驗，並系統性地嘗試以菌根接種方式，克服棄土石地之惡劣生育環境達到復舊造林目的。結果如下：煤礦棄土與石灰石礦棄土土壤性質殊不相同，前者呈極酸性(pH=3.05)且含鋁量極高而CEC低，後者呈鹼性(pH=8.20)且鈣含量極高；對兩種試驗地的自然植生與菌根感染調查，結果發現在所有自然植生者皆屬草本或矮灌木，在煤礦區者有5種植物，其中3種具有內生菌根感染，在石灰石礦區者有14種，其中9種是內生菌根；以菌根菌種而言分佈在煤礦區者有*Entrophospora colombiana*與*Glomus diaphanum*兩種，分佈在石灰石礦區者為*Acaulospora laevis, Glomus mossea*與*Gigaspora margartia*及兩種尚未鑑定之菌種，土壤中菌根菌孢子的分佈與植群覆蓋度及季節變化有顯著相關，高的值群覆蓋度及春季含孢子數最高；選擇陽性先驅樹種包括赤楊、台灣相思樹、銀合歡，利用盆栽分別以兩種礦區棄土進行內生菌根接種試驗，證實在溫室中內生菌根能夠幫助幼苗在煤礦與石灰石礦棄土中生長，惟野外試驗結果卻發現，石灰石礦棄土地內生菌根不但能促進苗木成活(平均增加15%成活率)，並可增加苗木生長(平均增加270%高生長)，但在煤礦棄土地接種內生菌根僅能延長初期的苗木成活率，經一年後所有苗木皆枯死，顯然內生菌根並無法克服煤礦棄土地的瘠劣地況；改用外生菌根菌*Pisolithus tinctorius*接種琉球

松造林於煤礦棄土地則可獲得極佳的生長，且對該棄土地土壤水、土壤化學與土壤生物具有改善作用。

八、英文摘要：

Mining activities could result in severe impacts on the conservation of soil and water resources. These adverse effects of mining are particular evident in Taiwan where rugged topographies and heavy rainfall characterize the environmental settings of this subtropical Island. The physical and chemical properties of spoils resulting from mining activities are extremely poor; establishment of trees on mining spoils, hence, is very difficult. The purpose of our project, therefore, was to study the feasibility of establishing trees on mine spoils of coal and limestone. Particular emphasis was made on the use of mycorrhizal manipulation to reforest the highly stressed spoils piling sites. Our results showed that there is a significant difference in properties between mine spoils of coal and limestone. The former is highly acidic (pH: 3.05) with very high aluminum content and low CEC whereas the former is slightly alkaline (pH: 8.20) with very high calcium content. Five and fourteen volunteer species were found, respectively, on coal and limestone mining spoils. Two of the former and five of the later are non-mycorrhizal; the rest are all associated with endomycorrhizae. Most of the endomycorrhizal fungi spores found in the coal mine spoils are *Entrophopora colombiana* and *Glomus diaphanum;* Whereas in the limestone mining sites *Acaulospora laevis, G. mossea, Gigaspora margarita* and two other unidentified species were discovered. Significant correlations were found between the distribution of mycorrhizal spores and the vegetation coverage. Spore distribution also shows very high seasonal variation and the spore count is the highest in spring. Greenhouse experiments showed that the performances of seedlings of selected tree species, *Alnus formosana, Acacia confusa* and *Leucaena leucocephala,* cultivated in mine spoils and inoculated with identified mycorrhizal spores are better than those maintained under the same conditions but without mycorrhizal inoculation. The field tests showed that in the limestone spoils survival rates as well as the growth of inoculated tree seedlings are 15% and 270%, respectively, better than uninoculated ones. In the coal mine spoils seedlings of all three species, inoculated or uninoculated, showed high mortality during the first two years after out planting; and none of them survive after the third years. It is concluded, therefore, that inoculation of selected tree seedlings with endomycorrhizaae dose not help improving the survival and growth of these species in harshed environment of coal mine spoils. Seedlings of *Pinus luchuensis* inoculated with *Pisolithus tinctorius,*

however, was found to be promising materials, in terms of both survival and growth, for establishment in coal mine spoils. The pine trees thus established have already shown their effectiveness in amending the soil properties of the coal mine spoils.

九、已發表報告目錄：

1. 周文能 1990. 煤礦區廢土石地自然植生菌根研究與松苗栽植試驗。國立台灣大學森林研究所碩士論文。
2. 顏江河 1996. 彩色豆馬勃琉球松菌根在煤礦棄土對土壤容液中鋁、硫含量及其吸收之效應。國立台灣大學森林研究所博士論文。

一、計畫名稱：

中文：線性規劃及模糊目標規劃在森林經營計畫決策上之應用

英文：An application of linear programming and fuzzy goal programming to the forest management planning and decision-making

二、執行期限：1993年7月1日至1996年12月31日止，共3年6個月。

三、執行單位：國立臺灣大學森林系

四、美方合作單位及人員：美國華盛頓大學森林系Bruce B. Bare教授

五、中方研究人員：楊榮啟

六、計畫目的：

近年來，臺灣的森林資源經營已經將重點放在以整體森林生態系為考量的森林生態系經營上。然而，林木收穫預測及森林生態系內部的交互作用，卻會使森林資源的經營管理環境產生不確定的因子。因此，臺灣大學與美國農業部林務署進行合作，希望能針對森林資源經營管理的不確定因子進行研究，以發展並改進森林資源經營的方法。

現今絕大部份森林資源的經營管理問題都是經由線性規劃方法進行解決，即使是多元化利用的森林資源經營問題也不例外。然而，線性規劃模式尚須要將不確定的因子納入模式中，究其原因，主要有三：

(一) 森林的經營範圍廣闊且經營時期長，森林資源的經營計畫往往延續數十年；

(二) 大部分的森林都包含了許多細小的林分，這些細小的林分各具有不同的生態環境，它們對社會提供的用途、價值、以及服務也不相同；

(三) 森林資源的經營過程中常受人為的干預及政策制定的影響，具有不確定性。

作者有鑑於此，發展一套可供解決不確定性資訊的數學決策方法，以助於未來經營管理的規劃，也是為整合森林資源經營管理的必然要求。

在過去，曾有學者建議利用幾項方法來解決森林不確定因子的問題。其中之一便是參數規劃方法。這個方法可以用來測試線性規劃模式中的一個或數個參數改變時，線性規劃模式最適解的改變情形。另一種方法則稱為機率或隨機規劃方法，它有助於解決逢機產生的不確定性的問題，然而具有其它不確定性的問題，靠隨機模式則無法獲得有效的解決。因此，本研究的目的，首先是針對某項資源永久收穫和環境保育之間的衝突，提供緩和之道；其次，發展能將不確定性因子納入考量的模糊多目標線性規劃模式。

七、執行成果摘要：

在現實世界中，對於事物所做的評估及判斷，多半是模糊而不是在清晰狀態下進行。實際上森林資源管理計畫方法的投入因子(如林地面積、林木蓄積、人力、經費等)及產出因子(如主產物、副產物、森林遊樂、水源涵養、野生動植物等)的數量，

也是屬於一種模糊狀態，因此可以應用模糊目標規劃理論及方法。

第一年研究之求解原理，是針對非模糊目標計畫模式內之數個目標函數式，選擇其主目標式為目標函數式，而將次要目標設為目標限制式，然後應用一般線性規劃方法求解多目標規劃問題之解。應用實例為國立臺灣大學實驗林溪頭營林區之案例，試行使用線性規劃方法求解模糊數學模式，並將其擴展到求解多目標規劃方法之不等權重問題上。

第二年度對問題之求解技術是先用一般線性規劃法來求解限制式下各個目標之極大值或極小值，據以客觀地決定各個目標的上下界目標值。再應用三角形隸屬函數，列出各個目標之隸屬函數式，並編擬成目標限制式而納入限制式中，以求解在最大隸屬函數下的結果，此結果可謂模糊目標規劃之最佳方案。而求解方法是接受美方學者Bare教授等人研究美國林務署多目標規劃問題所推崇之方案產生模式(MGA)，其求解的演算步驟是採納HSJ(Hop, Skip and Jump)方法：以求得之模糊目標規劃結果作為HSJ方法之起始解(initial solution)，觀察起始解中非零之決策變數，根據HSJ方法將此非零解加總成一新目標函數式，以求此目標函數式之極小值。在考量現實狀況下以三角形悲觀隸屬函數將此目標函數式模糊化，再次求解所有目標之最大隸屬函數值，以獲得一個新的可行解或可行方案，之後再觀察此一新方案中有無新增非零之決策變數出現；若有，則再將其併入原目標函數式中，並再次將其模糊化與求解；若無，則方案產生模式法則停止。這種方法是在模糊理論架構下重複運用模糊目標規劃演算法求解多重方案，能提供決策者更寬廣的決策空間。本年度以林務局所轄八仙山事業區資料作為研究案例，研究重點是試圖將現實的林業資料配合多目標規劃模式，據以統合為計畫模式。計畫模式之編擬，決策變數之設定是關鍵因素，須視林區經營目標及其擁有資源之資訊狀況而異，因此無從全盤接納國外範例，而須略施學術本土化的修正。

第三年繼續與美國林務署土地經營管理計劃組及華盛頓大學合作研究，期望能在臺灣落實研究成果，並從林業經營觀點對當前森林資源多目標利用與生態系經營之理念統合出本省林業可行的經營理論及方法。因此，本年度之規劃方法除了綜合前兩年度的研究理論及方法外，在模糊線性規劃中特別設定一個模糊參數區間h(0,1)用來表示決策者對於模糊數字的樂觀程度或確定程度。當h=1時，其效果為確定，則模糊模式不再產生任何作用；h<1時，其效果為不確定，則模糊模式隨即產生任何作用。同時為了能儘量符合現實之經營管理狀態，使環境因素對各個決策基本單位之影響程度降至最低，採用目標值(Zt或Zw)以代替各個決策變數加總之方法。本年度以林務局所轄臺東林區資料作為研究案例，研究重點是將森林資源聯合生產經營管理之理論及方法納入經營規劃的考量中，兼顧環境保育與經濟需求兩大目標能達到預期的結果。在產生經營管理方案的過程中，決策者可先不考慮處分面積大小，而

由產生的數個方案時，再以處分面積作取捨選擇出最適的方案，可以使環境保護與經濟利益二者衝突減到最小，達到林地水資源涵養能力受破壞程度降至最低之情形下，材積收穫量亦能符合經營計畫之預期目標。

八、英文摘要：

The growing intensity and variety of demands on forests for recreation, aesthetic and environmental benefits as well as timber give rise to complicated problems of the allocation of multiple-use in national forest around the world. Multiobjective programming has been considered as the best way for trading off the multiple-use of forest management in historical context. The applicability of this method allows specifying forest management problem in terms of multiple conflict objectives and allocating the forest multiple-use which is subjected to constraints and priorites. Furthermore, forest management is an integral part of the complexity and uncertainty of ecosystem interaction with the exception of the assumption of the coefficients of the yield model that are to be known with certainty when forest regulation problems are solved by deterministic LP models. Therefore, the developing fuzzy approaches are suitable to forest planning. This research developed a feasible method to overcome the imprecision coeffecients of optimization models. A solution approach based on Fuzzy Goal Programming is submitted. A feasible case in National Forest is presented.

九、已發表報告目錄：

1.楊榮啟、林文亮。1994。線性規劃及模糊目標規劃在森林經營計畫決策上之應用 中美農業科技第七期合作研究計畫(第一年)研究報告 46pp。

2.楊榮啟、林文亮、顏廷穎。1995。線性規劃及模糊目標規劃在森林經營計畫決策上之應用 中美農業科技第七期合作研究計畫(第二年)研究報告58pp。

3.楊榮啟。1995。模糊決策環境下之森林資源經營管理計畫 國立臺灣大學農學院實驗林研究報告9(4)：1-17。

4.楊榮啟、林文亮。1996。線性規劃及模糊目標規劃在森林經營計畫決策上之應用 中美農業科技第七期合作研究計畫(第三年)研究報告 58pp。

一、計畫名稱：

中文：研發對環境安全有效之住宅與商業用木製品抗燃劑

英文：Development of effective and environmentally safe fire retardant for wood products used in residential and commercial buildings

二、執行期限：1995年10月1日至1998年6月30日，共三年。

三、執行單位：台灣省林業試驗所森林化學系

四、美方合作單位及人員：

USDA, Forest Service, Forest Products Laboratory ;

Dr. G .C. Chen

五、中方研究人員：鄒哲宗

六、計畫目的：研發木製品用抗燃劑且研究抗燃藥劑處理過木製品之熱裂解機制。

七、執行成果摘要：

由熱重量分析圖及示差掃瞄熱分析圖，可見磷酸銨鹽類防火藥劑處理之柳桉合板，在170、210及270℃分別發生主要熱分解，而未處理之柳桉合板在270至320℃產生熱分解，這是因磷酸鹽促進纖維素脫水反應，顯著降低纖維素材料熱降解溫度之效應。此類防火藥劑處理木質材料具有良好之性質，不易起潮濕且有效期長，作為住宅與商業用木製品之抗燃劑十分可靠。

研製成具抗燃性纖維板，把其氧氣指數試驗結果，經專家設計分析，得其試驗方程式為：

LOI=20.15+0.704*(A:硬化劑量)+4.05x10^{-2}*(B:壓板溫度)+0.551*(C:抗燃藥劑量)+0.35*(D:壓板時間)

由此可判斷最大影響各板之最低需氧指數(LOI)值是抗燃藥劑量，其次為膠的硬化劑，再次為壓板溫度及時間。

八、英文摘要：

The addition of ammonium phosphates significantly reduces the thermal decomposition temperatures of Lawan plywood as indicated by DSC and TGA analysis at 270 and 320℃,whereas the treated sample took place at 170,210 and 270℃. It appears the phosphate of cellulose materials, and may be suitable as flame-retardant chemical for a variety of wood product applications. The treated materials have many advantage, including dimensional stability and long effectiveness.

It is to make fire-proof fiberboards through R&D, make the results of its Oxygen Index test be run by the Expert-Design Analysis, and obtain its test formula as follows：

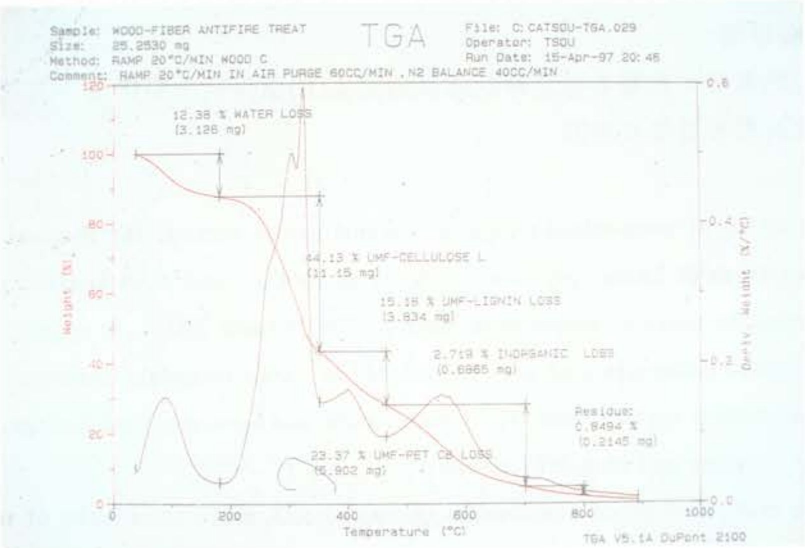
LOI=20.15+0.704*(A：Amount of Hardener)+4.04x10^{-2}*(B：Temperature of Board Pressing)+0.551*(C：Amount of Fire Retardant)+0.35*(D：Time of Board Pressing).

From this, it can be judged that the factor maximally affecting the Limited Oxygen Index (LOI)of various boards is the amount of fire retardant, followed by the amount of hardener for the colloid, and the temperature and time of board pressing.

九、已發表報告目錄：尚未發表

圖一、熱重量分析儀操作情形。

Fig. 1. The operation of thermogravimetric analysis.



圖二、抗燃纖維板之熱重量分析圖譜解析。

Fig. 2. The TGA thermogram of fire-proof fiberboard.

圖三、抗燃纖維板之最低需氧量試驗。

Fig. 3. The LOI experimental test of fire-proof fiberboard.

一、計畫名稱：

中文：國有人工林永續生態系經營的研究

英文：Development of sustainable management for plantation in national forests

二、執行期限：1993年7月1日至1996年6月30日，共三年。

三、執行單位：林業試驗所

四、美方合作單位及人員：

Pacific Northwest Forestry Research Station,

P. W. Owston, C. Y. Li.

五、中方研究人員：洪富文、游漢明、馬復京、張乃航、許原瑞等。

六、計畫目的：研發森林生態系經營在台灣進行的可行性。

七、執行成果摘要：

森林生態系經營對於長期的木材生產及生態系穩定的維持非重要。我們以地景生態學的概念為基礎，將竹東造林中心區視為全台灣森林區基質的一部分，並經過對此區內森林生態系重要組成分的調查，規劃出一個堅實的森林生態系經營計劃，以期達成永續經營的目標。

在1993～1994年，完成土壤及林分調查，並在幾處人工林及天然林林分內分析其組成、結構及土壤性質。1995年則完成經營規劃，並在1996年進行兩項符合生態經營原則的示範性處理。

竹東造林中心區總面積為8,200公頃，其中以柳杉為主的人工林有5,200公頃，10～30年生柳杉人工林密度為1,100～1,500株/公頃。天然林上冠層樹種有27～32種/公頃，其下層林木有16～17種/公頃，數量為20,000株/公頃。人工林下層林木有5～13種/公頃，數量為8,000株/公頃。天然林表層30公分土壤的有機物含量為人工林的2～3倍。

由於本區山谷陡峭，河岸帶不易到達，大多森林均為天然林，少受人為干擾，因此，形成良好的濱水保護帶，不需進行任何處理。

因此，本研究提出的生態系經營計劃重於10～30生人工林的疏伐，以改進其林分結構並促進下層林木生長，促進林分的生物多性，在比較老的人工林分，則以其受風害或其他為害形成的孔隙，進行刈草及加植重要的原生樹，促進其更新。

由於受到行政作業的影響，二項示範性處理，延至1996年始能進行。第一項為在20年生人工林進行的疏伐作業，第二項為50年生林分的孔隙更新作業。此二項處理後的植群及土壤的變化將持續進行監測。這類作業將可運用於台灣國有人工林造林中心區的經營作業，以達成生態系經營的目標。

八、英文摘要：

A sound forest ecosystem management is fundamental for achieving timber production, as well as for maintaining ecosystem stability in a long-term period. Based on

a landscape ecology concept, the Chu-Tung plantation center was perceived as a part of matrix in the whole forest area of Taiwan, and a management plan for fulfilling the goal of a sound forest ecosystem management was proposed after the study of current status of important ecosystem components of these forests.

In the beginning, the soil survey, and stand inventory were conducted, and stand structure and composition, and the soil properties of selected plantations and natural hardwood forests were analyzed in the first two years (1993-1994). Then a general forest management plan was developed in the third year (1995). And two pilot treatments were applied at stand level in plantations in the fourth year (1996) to demonstrate for the ecologically sound management practices.

The total area of Chu-Tung plantation center is about 8,200 ha with 5,200 ha of plantations dominated by pure *Cryptomeria japonica.* The stand density between 10-30 years of age was 1,100-1,500 trees/ha. In natural hardwood forests, species reached 27-3 2 species/ha in the upper canopy. The understory woody species of them was 16-17 species /ha with about 20,000 seedling, but only 5-13 species/ha with 2,000-8,000 seedlings in the plantations. As to the soils, the major difference between natural hardwood forests and plantations was organic matter content. In the upper 30 cm depth of the soils, the organic matter content in natural hardwood forests was 2-3 times higher than those in plantations.

A landscape view indicated most stream banks are steep valley slope areas, and covered with natural hardwood forests, which in turn form an almost intact riparian protection network that unaccessible for harvesting. Therefore, there is no need to do any treatment.

According to the current status, the proposed management plan will emphasize on the thinning of 10-30 years old plantations to improve their structure, and to increase their species diversity by stimulating the growth of understory hardwood seedlings. For gaps formed by wind damage or other causes in the older stands, weedings and enrichment planting of hardwood seedlings will be implemented to accelerate stand regeneration.

Because of the delay by some administrative regulations, two demonstrative treatments could be conducted only after a long overdue in the fourth year. The first one was a 5 ha thinning of a 20-year-old plantation, and second one was a 10 ha gap regeneration improvement in a 50-year-old plantation. The vegetation and soil dynamics of these two demonstration sites will be monitored, and adjusted according to adaptive management. these practices can be used for future forest management for national plantation centers.

九、已發表報告目錄：

1. 洪富文、夏禹九、張東柱、劉一新　1995.　竹東造林中心區森林生態系經營的規劃　p.213-220，洪富文等(編)　林業試驗所百週年慶學術研討會論文集　台北，台灣。

2. 程煒兒、洪富文、林光清、杜清澤　1995.　竹東造林中心區主要土系之理化性質及土壤養分經營　林試所研究報告季刊10:471-483。

中美農業科學合作研究計畫執行結果報告

Finaal and Progress Reports of COA-USDA Agricultural Science Cooperative Projects

漁業領域 (Fishery)

一、計畫名稱：

中文：草蝦生殖生理及成熟操控之研究

英文：Reproductive physiology and controlled maturation of tiger prawns, *Penaeus monodon.*

二、執行期間：1986年7月1日至1989年6月30日，

1991年7月1日至1992年6月30日，共4年。

三、執行單位：國立台灣大學漁業科學研究所

四、美方合作單位及人員：

Bodega Marine Laboratory, University of California, Davis, U. S. A.

Dr. Wallis H. Clark, Jr. and Ernest S. Chang,

五、中方研究員：郭欽明

六、計畫目的：探討對蝦類生殖生理調控機制，開發控制產卵技術

七、執行成果摘要：

對蝦類繁養殖技術之開發成功，促成產業的快速成長，在台灣水產養殖產業發展上至為重要，但生殖生理之基礎研究，並應用於產卵操控之技術開發仍有待加強之處。

蝦類眼柄中的X器官－靜竇腺分泌卵黃生成抑制激素，調控生殖腺之發育成熟，也協調脫殼抑制激素之分泌，影響蝦類脫殼作用。本研究就卵巢外觀，卵細胞構造特徵，瞭解卵巢發育過程，建立發育成熟之時期。眼柄中卵黃生成抑制素之萃取，分離及鑑定，經以Sephadex G-25分離出四個波峰(N1～N4)，其中N4具有卵黃生成抑制激素之功能，其抑制係數(Inhibition Index)為57.63%。N4波峰以逆相高效液層分析法，分離出6個波峰(F1～F6)，F4波峰之卵巢發育指數為1.17，抑制指數則為65.38%，確定F4具有VIH之成分。藉眼柄剪除以誘發卵巢發育成熟之效果，建立對蝦類生殖之基礎資料，並比較Reserpine，Domperidone，多巴胺，血清動素促進調控卵巢成熟之效果。生物胺及其擷抗物對卵巢成熟之效果，顯示種間之差異性，與生殖週期之關連性相當顯著。與生殖週期相斥之脫殼週期及血淋巴脫殼素之組成成分及含量變化，均已建立。

八、英文摘要：

Development of controlled reproduction and artificial propagation technologies is considered to be the key to the success of rapid development and continued growth of shrimp culture industry in Taiwan. The industry has significantly contributed to the economical development of the country. To date, basic research on the reproductive physiology aimed to develop controlled maturation and breeding techniques for penaeids hrimps is still remained to be emphasized.

Vitellogenesis inhibiting hormone (VIH) synthesized and released from X- organ Sinus gland complex plays an important role in regulating gonad development and maturation, and also influencing on the molting cycle through the regulation of molting inhibiting hormone (MIH). The ovarian development and maturation stages were determined by gross appearance of ovaries and cytological pecularities of ovarian oocytes. Fractionation and purification of VIH from eyestalks were also attempted. N1-N4 fractions were detected by gel filtration column chromatography (Sephadex G-25), and N 4 fraction containing VIH component with an inhibition index of 57.63%, was further subjected to RP-HPLC fractionation, by which VIH was detected in 4th fraction with 1.1 7 in the ovarian development index and 65.38 % in the inhibition index.

The effects of serotonin, dopamine and dopaminergic antagonists (domperidone and reserpine) were further examined , and the results suggested that the responses of the shrimps to these biogenic amines and drugs varied with species and reproductive phase as well. Finally, the changes in the morphological characteristics and hemolymph ecdysteroidal profile in *Penaeus monodon* were documented.

九、已發表目錄：

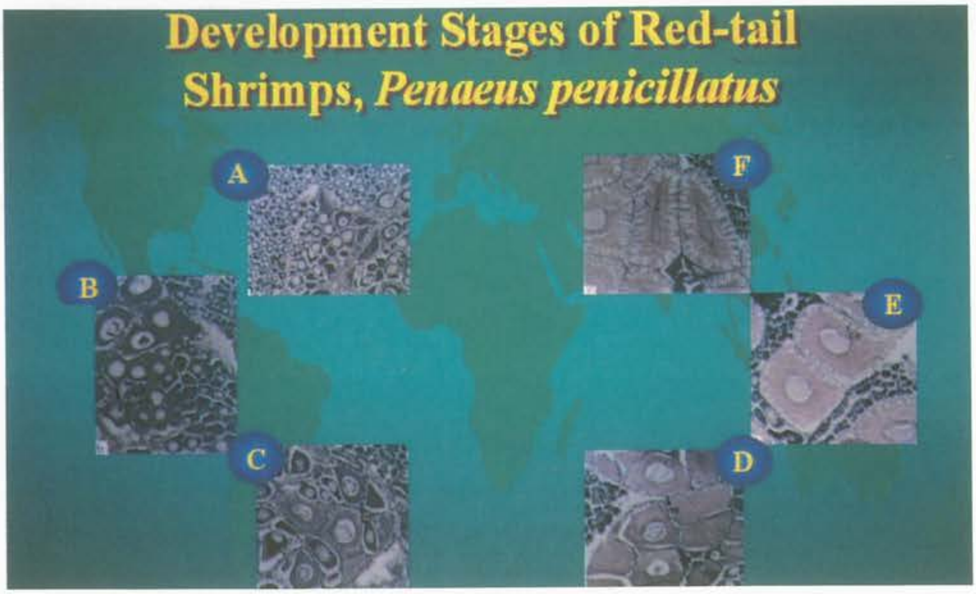
Kuo, C. M. and W. W. Lin. 1996. Changes in morphological characteristics and ecdysteroids during the molting cycle of tiger shrimp, *Penaeus monodon.* Zoological Studies, 35(2):118-127.

表一、多巴胺對紅尾蝦卵巢發育之效果比較

Table 1. Effects of dopamine on ovarian development in red-tail shrimps, *Penaeus penicillatus*.

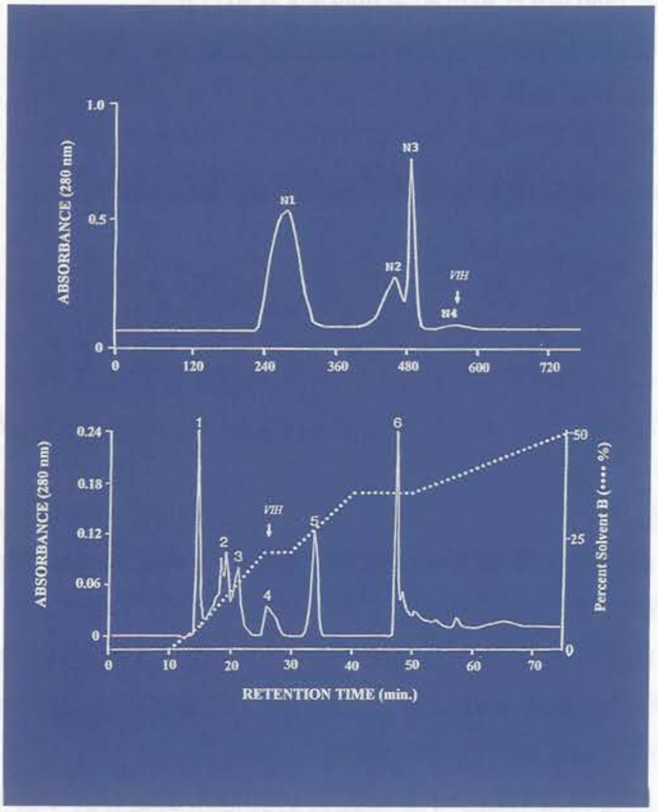
Effects of Dopamine on Ovarian Development in *Penaeus penicillatus*

Treatment	Number	Eyestalk	GSI	ODI
Saline	4	+	1.27 ±0.21	0
Saline	4	-	2.64 ±0.92	1.75 ±1.08
DA(2 μg)	3	-	3.14 ±1.23	3.67 ±0.90
DA(10 μg)	4	-	4.85 ±1.07	3.00 ±1.00
DA(40 μg)	3	-	4.74 ±1.79	2.67 ±0.58



圖一、紅尾蝦卵細胞發育過程

Fig. 1. Development stages of red-tail shrimps, *Penaeus penicillatus.* A: oogonia stage; B & C: previtellogenic stage; D: Yolk vesicle stage; E: Yolk globule stage, and F: Cortical alveolus stage.



圖二、草蝦眼柄中卵黃生成抑制素之分離，凝膠過濾層析譜(上圖)，N4 峰經以逆相高效層析儀解析(下圖)。

Fig. 2. Fractionation of vitellogenesis inhibiting hormone from the eyetalks of tiger prawns, *Penaeus monodon* by gel filtration chromatography (upper panel), from which N4 fraction was further subjected to reverse-phase high performance chromatography (lower panel).

一、計畫名稱

中文：水車對魚池動力學影響之研究

英文：The effects of paddlewheel on pond dynamics

二、執行期限：原定1986年9月30日起至1989年8月30日止，

提前於1988年8月30日結束。共進行2年。

三、執行單位: 中山大學海洋生物所

四、美方合作單位及人員：

The Oceanic Institute, Makapuu Point, Waimanalo, HI 96795, USA

Dr. Gary Pruder

五、中方研究人員：方新疇、方力行、陳一鳴、陳宏遠、李玉玲

六、計畫目的：

探討水車之運作對鹹水蝦池中之物理,化學及生物因子之影響，及如何利用這些因子與水車的交互作用,以增加其生態負載量，及維持蝦池養殖動力結構的穩定性。

七、執行成果摘要

由1987年至1988年間，利用水產試驗所台南分所的鹹水草蝦養殖池從事試驗。除了以不同組合的水車數目和放養率進行養殖試驗之外，並且就物理，化學和生物方面作短期性之監測探討。

前後兩期的養殖池試驗因為受到了當時嚴重流行之草蝦病變影響。美方要求把第三年之研究移至夏威夷繼續進行。台灣方面之學者因教學工作上的責任無法配合，決定停止。目前列出者為前兩年的試驗所得之成果。

水中溶氧的監測結果顯示水車之運轉對於池水氧氣有若干影響。在夜間缺氧時可發揮打氣機之作用,但在白天則作用有限。氧氣在過飽和時則可予以降低。基本上其打氣的功效很容易被其它因子，如水溫或光照所掩蓋。但由於水車的持續運轉造成池水大規模混合，對於基礎生產量，營養代謝甚至蝦苗攝食動物性浮游生物都可能有間接促成的作用。在固定位置持續運轉的水車對於池底泥土之顆粒分布狀況和不同區域之沉澱速度均有顯著之影響。

長期性養殖實驗損失超過1/3池數。統計分析結果顯示水車數目之多寡對於生產量增加或降低草蝦池發病率均無顯著之影響。

綜合而言水車在草蝦養殖中可發揮多用途之功能,但其作用短暫,除底泥顆粒分布狀況為長期性之外其餘均無法累積。在其作用範圍內可視為維持養殖系統穩定之工具。

本研究中並且由系統生態學的角度對於蝦池的環境因子作了概括性的探討。結果顯示決定水中溶氧量變化之主要因子除了晝夜不同外尚包括光照，酸鹼度，水溫，養殖生物 (蝦) 總重量等。 其長期性效果均較水車數目顯著。

八、英文摘要

The object of this project was to understand the effects of paddlewheel on the physical, chemical and biological factors in shrimp ponds, and how to access these factors and the interactions resulting from various paddlewheel operation strategies, in order to increase carrying capacity and/or stability of pond dynamics.

In 1987 and 1988, pond experiments were conducted to study the effects of paddlewheels on shrimp production under different stocking rates. Also, experiments were set up for monitoring various ecological factors. Toward the end of 1987, stocks in 2 of the 12 ponds were lost under the the presssure of shrimp disease. Next year's experiment with higher stocking rate suffered more. Seven ponds among 12's were again lost. The funding agent had decided to terminate the project in Taiwan and requested that the last years experiment be moved to Waimanalo, Hawaii. However, the scientists in Taiwan found that it was impossible for them to leave their teaching duty and conduct experiment in Hawaii. The project were thus terminated by the end of 1988.

The publications listed represent the results of the efforts between September 1986 and September 1988.

Study of dissolved oxygen showed that paddlewheel was not a powerful equipment whenever aeration is conscerned. The aerating effect of paddlewheel was easily masked by factors such as light intensity and temperature.

Nevertheless, operation of the paddlewheel does increase low dissolved oxgen at night, and decrease oversaturation during the day. Continuous flowing water resulted in an overall mixing effect. Possible secondary effects included: more effective primary productivity, metabolism of nutrients and promotion of zoolplankton predation by juvenile shrimps. For the pond with paddlewheel(s) operated at fixed positions, particle distribution on the bottom and rate of sedimentation were significantly affected.

Statistical analysis on the results of the cultural experiments demonstrated a significant relationship between production and shrimp stocking rate. The effect of paddlewheel on shrimp production as well as preventing shrimp disease were not significant.

In conclusion, the role of paddlewheel on shrimp culture is multipurpose but mostly temporary. The only exception is the sedimentaion pattern on the bottom soil. It acts as an stabilizer that somehow maintained, within its own limits, a constant environment for beter shrimp production.

Other contribution of this project includes a systematic study of factors affecting dissolved oxygen of the shrimp pond.

The project also benefited in funding from the Council of Agiculture, ROC (Grant No. 77-Agr.Const.-71-Fisheries 23-8).

九、已發表報告目錄：

1. Fong, S. C. 1990. The effects of paddle wheels on the physical environments of shrimp ponds. In: R. Hirano and I. Hanyu. (eds.) 1990. The second Asian Fisheries Forum 991p, Asian Fisheries Soc., Manila. pp121-124.
2. Fong, S. C., T. L. Chen and C. W. Chen. 1990. An automatic system for monitoring temperature and dissolved oxygen content within fish ponds. J. Fish. Soc. Taiwan. 17(4): 81-87.
3. Fong, S. C. 1990. Simulation and system analysis of dissolved oxygen content in earthern ponds. J. Fish. Soc. Taiwan. 18(1):47-54.
4. Fong, S. C., H. Y. Chen, Y. Y. Ting, and M. H. Chang. 1991. Effects of paddlewheel and stocking density on the production of tiger prawn, *Penaeus monodon,* in saline ponds. J. Fish. Soc. Taiwan. 18(4):279-286.
5. Huang, J. C., I-M. Chen and L. S. Fang. 1990. The role of organic molecules in controlling the water quality of shrimp ponds. In: R. Hirano and I. Hanyu. (eds.) 1990. The second Asian Fisheries Forum 991p, Asian Fisheries Soc., Manila. pp113-116.
6. Huang, J. C., I-M. Chen and L. S. Fang. 1990. The effect of a paddlewheel on the nutrient dynamics in a shrimp pond. In: R. Hirano and I. Hanyu. (eds.) 1990. The second Asian Fisheries Forum 991p, Asian Fisheries Soc., Manila. pp125- 128.
7. Lee, Y. L. and H. Y. Chen. 1990. Effects of paddlewheels on daily fluctuations of dissolved oxygen concentration in shrimp ponds. In: R. Hirano and I. Hanyu. (eds.) 1990. The second Asian Fisheries Forum 991p, Asian Fisheries Soc., Manila. Pp117-120.
8. Lee, Y. L. and H. Y. Chen. 1991. Juvenile *Penaeus monodon* as effective zooplankton predators. Aquaculture, 103(1992) :35-44.

一、計畫名稱：

中文：養殖草蝦之蛋白質需求與消化研究

英文：Protein requirement and digestion in cultured *Penaeus monodon.*

二、執行期限：1987年7月1日至1992年6月30日，共5年

三、執行單位：海洋大學，中山大學

四、美方合作單位及人員：

Mississippi State University.

Dr. Louis Debramo

五、中方研究人員：孫寶年、蕭錫延、陳宏遠

六、計畫目的：

1. 瞭解養殖草蝦之基本蛋白質消化酵素之特性。
2. 瞭解養殖草蝦之營養需求。
3. 確定草蝦蛋白質之需求量及蛋白質消化酵素間之關係。
4. 建立草蝦飼料中蛋白質與能量間之正確比例。
5. 建立正確完善的養殖草蝦人工配合飼料之基本資料：

台灣草蝦養殖自1968年人工繁殖成功以來獲得極大之進展，養殖數量也大幅上升，至1985年冷凍草蝦出口已達14,000噸，價值超過美金一百三十萬元。雖然，草蝦養殖技巧在台灣建立了及好的聲譽，但是其基本之營養需求量卻一直沒有系統的研究。本研究計畫為研究草蝦對蛋白質之基礎實驗以瞭解草蝦對蛋白質之利用性以為改善草飼料之品質，降低成本之參考。

七、執行成果摘要：

草蝦(*Penaeus monodon*)飼養於鹽度36ppt之海水中，以蛋白質含量為36 %之飼料飼養，當飼料熱量增加至330kcal/100g時，發現飼料轉換率、增重率及蛋白質增加均有改善；蛋白質含量為40%之飼料時則以熱量320kcal/100g之飼料較佳。若飼養於鹽度16ppt之海水時，以含44%蛋白質之飼料餵食會有最大增重率，而鹽度32ppt時則為蛋白含量40%之飼料。精胺酸需要量為25g/kg飼料。澱粉為最好之碳水化合物來源，其次為糊精，再其次為葡萄糖($p<0.05$)。

由攝取飼料之停滯時間，組織pH，及蛋白　活性在前腸、中腸腺、中腸及後腸等部位之體內試驗發現蛋白質消化主要在中腸腺進行，先經pH 7.5 之內切型胜肽酶消化，再由pH 4.5之外切型胜肽酶進行兩段pH式消化。體外模擬草蝦腸道蛋白質消化速率，顯示豐年蝦最易被分解，其次為經加熱預溶之酪蛋白，再依序為麵筋、造粒草蝦飼料、未造粒草蝦飼料、紅魚粉、白魚粉、黃豆粉、及酵母粉。蛋白質消化速率與鹼性胺基酸含量呈線性關係。未經加熱處理蛋白質之消化速率隨其芳香族胺基酸含量之增加而增大，而熱變性蛋白質之消化速率卻隨之降低。

八、英文摘要：

Grass Shrimp, *Penaeus monodon* reared in seawater (salinity 36 ppt), showed improved feed conversion ratio, weight gain, and protein gain as dietary level was raised to 330 kcal/100g when dietary protein was 36%, or 320 kcal/100g when the latter was 40 %. Maximal weight gain and protein retention was 44% when culturing salinity was 16 ppt, and 40% at 32 ppt. The arginine requirement was 25g/kg diet. Starch was a carbohydrate source better utilized than dextrin followed by glucose ($p<0.05$).

In-vivo measurement of the retention time of feed ingesta, tissue pH, proteolytic enzyme activities in foregut, midgut gland, midgut and hindgut indicated the protein digestion took place mainly in midgut gland by endopeptidases followed by exopeptidases at two pH stages, 7.5 and 4.5 respectively. An in-vitro method simulating the in-vivo protein digestion was developed. Feed protein showed the following order of digestibility: Artemia > predissolved casein > gluten, pelletized shrimp feed > unpelletized shrimp feed > brown fish meal > white fish meal > soybean meal > yeast. Protein digestibility bore linear correlation with content of basic amino acids in feeds. Increased aromatic amino acid content increased digestibility of the feed protein, without previous heat treatment, but reduced the digestibility of the heat-treated protein source.

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一、計畫名稱：

中文：照射對草蝦肌肉微細構造及細胞化學的影響

英文：Ultrastructural and cytochemical effects of gamma radiation on grass shrimps (*Penaeus monodon* F.)

二、執行期限：1987年7月1日至1991年6月30日, 共4年

三、執行單位：食品工業發展研究所。

四、美方合作單位及人員：

Drs. George P. Hoskin and Clyde A. Takeguchi, FDA, USA.

五、中方研究人員：楊瑞森、彭芳琇、吳家駒、黃錦城、劉素娥

六、計畫目的：

探討草蝦經加馬線照射後，肌肉細胞微細構造及細胞化學(包括褐化酵素，蛋白分解酵素及鈣離子分佈)的影響，作為照射處理草蝦的品質評估參考。

七、執行成果摘要：

第一年工作：

草蝦在常溫或冷凍(-18℃)狀態下經加馬線照射後，以掃描式電子顯微鏡和穿透式電子顯微鏡來觀察其腹部肌肉微細構造受照射影響情形，結果顯示，以2或5 kGy照射後當天和照射後放置於4℃以下冷藏8天的草蝦肌肉微細構造並無顯著改變，但在常溫下以10 kGy照射後當天的草蝦，其肌肉纖維的I-帶(I-band)縱切面呈現出很不規則鋸齒狀，在橫切面上亦可觀察到A-帶(A-band)中的一些肌凝蛋白(myosin)和肌動蛋白(actin)消失，這能是由於在常溫下10 kGy加馬照射草蝦會立即造成腹部肌肉中A-帶的肌凝蛋白和肌動蛋白受到破壞，此外，肌肉中的粒線體和肌質網膜亦受到某程度損壞。

在-18℃下冷凍處理的草蝦，經10 kGy照射後，其肌肉微細結構仍完整，這顯示冷凍處理可以保護肌肉微細結構免於照射所造成的破壞。

綜合本實驗結果得知，在常溫狀態下以5 kGy照射或在冷凍(-18℃)處理下以10 kGy照射之草蝦,在微生物總量、組織風味和微細構造上評估的結果是令人滿意的。

第二年工作：

草蝦在冷凍狀況下經40 kGy加馬線照射後，觀察高劑量照射對肌肉微細構造及蛋白質水解酵素活性的影響。40 kGy冷凍照射後當天的草蝦肌原纖維中的肌凝蛋白、肌動蛋白、肌質網和粒線體都受到某程度破壞。經常溫存放三週後，以40 kGy照射後的全蝦的第一腹節肌原纖維已完全崩解且有多量體液流出。蝦體液與*Bacillus licheniformis*的蛋白質水解酵素具有類似分解草蝦肌原纖維的功能，而在蝦體內的蛋白質水解酵素活性受40 kGy 照射影響不顯著。因此，欲以高劑量冷凍照射處理草蝦來延長儲存期，首先必須以其他加工技術抑制蛋白質水解酵素活性。

第三年工作：

本計畫觀察加馬線照射對草蝦肌肉離子分佈、色素胞及揮發性成份之影響。草蝦在冰浴狀態下，經5 kGy加馬線照射，於4℃下草蝦貯存一星期後，有明顯的黑變產生，取其表皮層以光學顯微鏡觀察，結果顯示表皮層上色素細胞中的黑色素細胞有明顯擴散現象，故使體色呈黑色。5 kGy 照射並不能抑制草蝦的黑變和自體分解。以 pyroantimonate 沈澱的細胞化學方法製備肌肉組織的樣品，再以電子顯微鏡觀察和電子微探儀分析草蝦肌肉組織內的鈣離子分佈情形，5 kGy照射後當天和在4℃下貯存7天的草蝦肌肉細胞內鈣離子分佈情形並無顯著差異;未經照射的草蝦經4℃下貯存7天後，其肌肉細胞內的肌原纖維和肌質網之鈣離子濃度有明顯增加趨勢，此較肌肉經5 kGy照射的草蝦肌肉的自體分解速率快，是否有所關連？需待進一步的研究探討。

另以Likens-Nickerson 蒸氣蒸餾法萃取草蝦揮發性化合物,再以氣液相色層層析儀分析，發現5 kGy照射後的草蝦並未有明顯特異的含磷和含氮揮發性化合物產生。

第四年工作：

觀察5 kGy照射對草蝦肌肉、肝胰臟和表皮層內phenoloxidase分佈的影響。草蝦在冰浴狀態下，經5 kGy加馬線照射後，以免疫細胞化學方法處理，以phenoloxidase抗體與colloidal gold複合體，即免疫金染色後，再以uranyl acetate和lead citrate雙重染色後，經電子顯微鏡的觀察初步結果顯示，phenoloxidase多分佈在肌肉的肌質網、肝胰臟的內質網、脂肪油滴、細胞質、毛狀突起物緣和表皮層內的內質網、脂肪油滴、細胞核及色素細胞中。5 kGy照射處理不會明顯改變草蝦體內phenoloxidase分佈情形。

八、英文摘要：

Treatment with intermediate ionizing radiation (such as 5 kGy) is useful in elongating the refrigerated shelf life of the shrimps (Rothbart 1986). Although observations concerning nutritional and microbiological changes of irradiated shrimps are available (Kumta *et al.* 1970; Yeh and Hau 1988), extrapolation to cytoplasmic events still remain speculative.

The effect of ionizing radiation (2, 5 and 10 kGy of gamma rays) on muscle ultrastructure of grass shrimps (*Penaeus monodon* Fabricius) tails at ambient or frozen (-18℃) temperature was investigated by transmission electron microscopy (TEM). No significant change was found in muscle ultrastructure of shrimp meat irradiated at doses of 2 or 5 kGy and then stored at 4℃ for 0 and 8 days. However, in shrimps which were irradiated at ambient temperature at a dose of 10 kGy, the margin of A-band of the myofibrils in longitudinal sections exhibited an irregular zig-zag pattern. In cross section some myosin filaments in the A-band regions were missing. A possible interpretation of these results would be that 10 kGy gamma irradiation dose at ambient temperature

depolymerized myosin in the A-band from the tail of thick filament. Actin damage was also observed in some cross sections, irradiation damage of sarcoplasmic membranes appeared in the specimens irradiated at a dose of 10 kGy at ambient temperature. Shrimps frozen at -18℃ and then irradiated with 10 kGy did not exhibit filament damage. Freezing apparently protected shrimp meat from irradiation injury.

Ultrastructural changes occurred in the muscle of grass shrimps irradiated at the dose of 40 kGy were observed with electron microscopy. Frozen whole shrimps irradiated with 40 kGy immediately appeared damage of myosin and actin. Some broken mitochondria and sarcoplasmic reticula were observed in the irradiated shrimp muscle. On the other hand muscle from irradiated whole shrimps was seriously autolyzed and released a large quantity of body fluid after three week storage at ambient temperature.

The activity of proteinase extracted from isolated heptopancreas was not inactivated by irradiation and the enzyme still retained the same activity after three week storage at 4℃ as that of fresh shrimps. Myofibers were seriously autolyzed when shrimp muscle tissues were incubated with the crude enzyme from heptopancreas for 2 hours.

Therefore the case of irradiation to sterilize grass shrimps was limited by the residual activity of protelytic enzyme. Hence heat treatment should be combined with the irradiation process to inhibite proteinase activity.

Effects of medium-dose (5 kGy) irradiation on calcium distribution, chromatosomes and volatile components of shrimp muscle were studies by using electron microprobe (EDX) X-ray microanalysis with electron microscope and gas chromatography. In irradiated shrimp muscle cells calcium ions in sarcoplasmic reticula and nuclei were not released to fibril and still kept *in situ* one week after irradiation. However, without irradiation treatment the concentration of calcium in myofibril of muscle cells became significantly high. It was suggested that in shrimp muscle cells without irradiation treatment calcium ions were released from sarcoplasmic reticula and nuclei to myofibril. Medium-dose irradiation treatment could inhibit the dispersion of melanins. No significant change of volatile components was found after irradiation.

Effects of medium-dosse (5 kGy) irradiation of phenoloxidase distribution of grass shrimp (*Penaeus monodon* F.) was studied by using immunocytochemical method and electron microscopy. Phenoloxidase was isolated and purified from the shrimps. Antiphenoloxidase chicken antibody (IgG) was made and gold-anti-chicken IgG complex was prepared to stain shrimp ultrathin sections for localizing phenoloxidase. The enzyme was found and widely localized in endoplasmic reticula, lipid droplet and microvillar border

of hepatopancreas, endoplasmic reticula, lipid droplet and nuclei of epidermis and pigment cells. The distribution of phenoloxidase in shrimp after irradiation was compared by the immuno-gold technique with that before irradiation. No significant change of phenoloxidase distribution in shrimp was found after irradiation.

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一、計畫名稱：

中文：草蝦之品質維護與廢棄物之利用

英文：Keeping the quality of post-mortem grass prawn and utilization of the discards from processing

二、執行期限：1988年7月1日至1992年6月30日，共4年

三、執行單位：國立台灣海洋大學水產食品科學系

四、美方合作單位及人員：

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Dr. Michael W. Moody

五、中方研究人員：陳幸臣、江善宗

六、計畫目的：

本研究之目的為探討草蝦死後之生化學變化及細菌相改變，該類變化與冷凍草蝦之品質關係，進而改變其品質，以及草蝦頭之加工利用。

七、執行成果摘要：

本研究首先比較目前草蝦由養殖場到加工廠之運送方式對品質的影響，發現以揮發性鹽基態氮(VB-N)、TCA-可溶性氮(AA-N)及K-值結果來看，氧氣運送者比冰藏者要佳；但由核苷酸類化合物及微生物污染結果來看，兩者則無差異。本研究亦探討臭氧處理對蝦肉影響，結果顯示被測試九種菌中只有沙門氏菌具有抗臭氧性，同時在三十分鐘內能將大腸桿菌及Phage M13之DNA分解，由Ames Test證明經臭氧處理之蝦肉無致癌物質。進一步為瞭解冷凍草蝦肉質軟化與失重原因，由草蝦消化道純化四種蛋白酶，分別為trypsin-like及chymotrypsin-like蛋白酶，其最適pH在7.0～8.0間，與草蝦肉質分解有關連，草蝦肌肉之肌動凝蛋白(AM)安定性較虱目魚、吳郭魚及鯉魚者要低，變性速率隨凍藏溫度上升而加快，但在-30℃與-40℃凍藏時則無差異，意味著草蝦凍藏溫度宜在-30℃。由草蝦肌肉純化之組織蛋白酶在pH7.0～8.0間仍然很安定而且在pH 6.0下仍有活性，所純化之鈣肽酶卻在pH7.0～8.0時活性最高，足見冷凍草蝦肉質軟化與肌肉中存在之組織蛋白酶及鈣肽酶有極密切關係，由該類蛋白酶之作用溫度來看，草蝦加工作業宜保持在10℃以下。

本研究同時探討利用*Cellulomonas flavigena* NTOU 1水解草蝦加工後之廢棄物，結果顯示由*C. flavigena* NTOU 1所產生之變異株(type A及B) 對草蝦肉及殼均有高水解力，根據動物試驗結果顯示，變異株B所產生之水解物具食品安全性，可作為蝦醬油加工原料；該變異株B水解蝦頭及蝦殼之收率遠高於枯草菌及胃蛋白酶，由官能品評及氨基酸、脂肪酸與核苷酸之成分評估，結果顯示以*C. flavigena* NTOU 1B之水解液最佳；其風味可經由醬油酵母之發酵而獲得改進；水解後殘渣飼養小雞之飼料轉

換率與蝦頭粉相比則無差異(p>0.05)。

八、英文摘要：

The postmortem biochemical and bacteriological changes of grass prawn during transportation from aquatic farm to processing plant were investigated. According to the volatile basic nitrogen (VB-N), TCA-soluble nitrogen (AA-N) and K-value, shrimp transported by oxygenation was superior to that by icing. However, no distinct differences in nucleotide profile and bacterial contamination was observed between these 2 transportation methods. For investigating the effects of ozonative disinfection on shrimp, 9 bacterial strains were tested. *S. typhimurium* was found to be resistant to ozone, while the DNA of *E. coli* and phage M13 of outside bacterial cell were lyzed within 30 min of ozonation. There was no mutagens existed in shrimp meat, according to Ames test. Three trypsin-like and 1 chymotrypsin-like proteases were purified from grass prawn digestive tract and characterized. From the optimal pH of these enzymes, they were considered to be highly related to the degradation of shrimp meat. The thermostability of actomyosin (AM) from grass prawn seemed to be lower than that of milkfish, tilapia and carp. Inactivation rate of AM increased with the increase of storage temperature. However, no significant difference in KD was obtained between samples stored at -40℃ and -30℃. This phenomenon suggested that the optimal temperature for frozen grass prawn was -30℃. This study further purified and characterized the cathepsin D and calpain from shrimp muscle. Cathepsin D had stable pH around 7.0～8.0, and still had 30% activity around pH 6.0. The optimal pH for calpain activity was around 7.0～8.0. These characteristics suggested the involvement of these proteases in degradation of shrimp. According to optimal temperature for these enzymes, the temperature during processing should be kept below 10℃.

For utilizing the discards of shrimps during processing, mutants type A and B from *Cellulomonas flavigena* NTOU 1 revealed higher hydrolysis capacity on whole shrimp than other strains and proteinases tested in this study. From the animal test, the hydrolysates obtained by *C. flavigena* NTOU 1B was considered safe as a starter culture for shrimp sauce processing. Recovery of the hydrolysate by *C. flavigena* NTOU 1B was higher than that hydrolyzed by *B. subtilis* and pepsin. Hydrolysate produced by *C. flavigena* NTOU 1B was tested the best, judged by sensory evaluation, and profiles of amino acids, fatty acids and nucleotides. Flavor could be further improved by growing *Saccharomyces rouxii* in the hydrolysate. As an ingredient of chicken feed, no significant difference in feed conversion ratio was observed between the hydrolysate residue of

shrimp head and shell produced by *C. flavigena* NTOU 1B and then fermented by *S. rouxii* and the shrimp head powder (p>0.05).

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表一、草蝦肉於冰藏及氧氣運送期間核苷酸的變化。

Table 1. Changes in nucleotides of prawn meat during transportation by icing and oxygenating.

Treatment[a]	Determination	Transportation time (hr) 0	10	18	26
		Content (μmole/g)			
G-I	ATP	6.38	3.63	1.03	0.62
	ADP	2.18	0.57	0.49	0.67
	AMP	3.33	1.79	2.05	2.88
	IMP	6.88	6.98	7.77	7.41
	HxR	trace	trace	0.77	0.90
	Hx	trace	trace	trace	0.36
	IMP Ratio	100%	100%	91.0%	85.5%
	K-Value	—	—	6.4%	9.8%
G-II	ATP	6.38	1.05	0.82	0.00
	ADP	2.18	0.36	0.32	0.00
	AMP	3.33	2.47	2.26	1.48
	IMP	6.88	7.69	7.98	7.77
	HxR	trace	0.64	0.90	1.01
	Hx	trace	trace	0.12	0.40
	IMP Ratio	100%	92.3%	88.7%	84.6%
	K-value	—	5.2%	8.2%	13.2%

[a] G-I: Oxygenated prawns; G-II: Iced prawns.

表二、草蝦肉於冰藏及氧氣運送期間微生物的變化。

Table 2. Microbiological changes of prawn during transportation by icing and oxygenating.

Transportation time (hr)	Treat-ment[a]	APC (Log CFU/g)	*Pseudomonas* (Log CFU/G)	Fecal coliform (MPN/g)
0		5.392±0.241 A[b]	3.457±0.160 A	< 3
10	G-I	4.578±0.268 B	3.403±0.129 A	< 3
	G-II	5.038±0.190 A	3.404±0.141 A	< 3
18	G-I	4.611±0.270 B	3.461±0.332 A	< 3
	G-II	4.764±0.386 B	3.436±0.124 A	< 3
26	G-I	4.799±0.221 B	3.946±0.352 A	< 3
	G-II	4.746±0.149 B	3.443±0.073 A	< 3

[a] G-I: oxygenated prawns; G-II: iced prawns.

[b] Comparison between 0 hr and 10, 18, or 26 hr was done by t-test. Values bearing unlike characters in the same column differ significantly ($p<0.05$).

圖一、以臭氧處理的大腸桿菌JM107之M13RF DNA及噬菌體M13之M13 ss DNA的膠電泳圖。這些DNA均先混合於M9培養基，再以臭氧處理(100 mL/min)，使在30 min處理後之臭氧濃度的5 mg/L

Fig. 3. Gel electrophoretic pattern of ozonated M13 RF DNA in *Escherichia coli* JM107 and M13 ssDNA in phage M13. M13 RF DNA transformed-E. coli JM 107 and phage M13 were suspended in M9 medium which was ozonated (100 ml/min) to obtain a final ozone concentration 5 mg/L within 30 min. Lane 1, 2, 3, 4, 5, 6 and 7 indicated the pattern of M13 RF DNA after ozonating for 0, 5, 10, 15, 20, 25 and 30 min, respectively; lane 9, 10, 11, 12, 13, 14 and 15 indicated the pattern of M13 ssDNA after ozonating for 0, 5, 10, 20, 25 and 30 min, respectively; lane 8 indicated ladder marker. C: scDNA (supercoil form); B: ocDNA (open circular form); A: linear DNA.

一、計畫名稱：

中文：吳郭魚耐寒性之生理生化與育種之研究

英文：The biomedical analysis and genetics selection of to cold tolerance.

二、執行期限：1988年7月1日至1992年6月30日，共4年

三、執行單位：國立台灣海洋大學 水產養殖學系

四、美方合作單位及人員：

TVA F-137, NFDC Muscel Fhoals, Alabama

Dr. Leslie L. Behrends

五、中方研究人員：張清風

六、計畫目的：

(1)探討吳郭魚經低溫處理後，重要生理生化指標反應。

(2)探討吳郭魚經低溫處理後，不同時間的生理生化指標反應。

(3)探討經適應於不同鹽度與溫度對吳郭魚的低溫生理反應。

(4)比較吳郭魚與鯉魚對低溫的忍受與生理反應。

七、執行成果摘要：

低溫對吳郭魚的養殖是一個重要的問題，它造成吳郭魚的緊迫生理反應，若能由緊迫反應中尋得重要的生理生化指標，供為判斷吳郭魚是否遭受寒冷環境，對將來在做育種方面是相當重要之選種標準。有關一般魚類緊迫生理反應，包括三級反應：(1)神經內分泌反應 (2)一般生理反應 (3) 對成長、生殖、疾病與死亡等影響。因此本研究之目的為探討吳郭魚經低溫緊迫後，其重要生理生化指標之特性，並比較適應不同的時間、鹽度對低溫的反應。另外選擇鯉魚作為與吳郭魚比較對寒冷的耐性及生理生化指標之反應。由研究的結果得知血紅素僅在24小時(急速低溫，30-14℃，24 小時實驗組)反應中會增加，而血漿氯離子在62小時(緩慢低溫28-14℃，86 小時實驗組)會降低。血漿滲透壓、白血球容積比、乳酸鹽濃度在24小時及86小時實驗組均會降低。血糖濃度僅會在86小時實驗組增加。Cortisol 濃度在24小時實驗組會增加，而後在86小時實驗組則降低。由本實驗得知白血球容積比、乳酸鹽、血糖、cortisol 可供為判斷吳郭魚經低溫處理後的生理反應指標之一。接著，由養殖鹽度之條件下，會增加或降低吳郭魚不同的生理生化指標值，因影響的程度，依不同鹽度而有所差別。依其白血球容積比、紅血球容積比、氯離子、滲透壓變化及冷昏迷反應得知，適應於鹽度的吳郭魚比養殖於淡水較不耐寒冷。吳郭魚在冷昏迷階段時，血紅素、血球容積比、白血球容積比、血漿乳酸鹽濃度會顯著降低，但血糖、cortisol及鰓 Na^+-K^+-ATPase活性則反而顯著的增加。鯉魚顯示比吳郭魚耐寒，在遭受8℃水溫時，鯉魚之生理生化指標大多沒有顯著的改變，直到遭受到4℃時，血漿乳酸鹽、血球容積比、血紅素及白血球容積比才受到顯著的影響。

八、英文摘要：

Low temperatures are a problem for tilapia cultivation. Exposure to cold temperature is one of the environmental stressors which elicit various responses. Behavioral changes are useful indicators of upper and lower temperatures but one must rely more heavily on biochemical and physiological parameters for evaluation of sublethal stressors. The stress responses have been classically separated into three stages, neuroendocrine responses, physiological responses, the effects on growth, fecundity, disease susceptibility and behavior change. The objectives of this study were to characterize the stress responses in tilapia exposed to low temperatures. Common carp, a species tolerated to colder temperatures, was also selected for the comparison. The results showed that blood hemoglobin increased only in the 24 h experiment , plasma chloride decreased only after 62 h in the 86 h experiment. Plasma ormolarity, leucocirt and lactate decreased in both the 24 h and 86 h experiments. Glucose increased significantly only in 86 h experiment. Cortisol increased in the 24 h experiment and then decreased abruptly in the 86 h experiment. Salinity significantly caused the decreases of hemoglobin concentrations but the increases of lactate, chloride and osmolarity levels in tilapias cultured at 24 ℃ . Low temperatures have the significant effects of either increasing or decreasing direction on the change of various blood parameters in tilapias depending on the environmental salinity. Salt-acclimated tilapias are suggested to be less tolerant to the low temperatures on the basis of the data of hematocrit, leucocrit, chloride, osmolarity and comatose responses. Changes in blood parameters and gill Na^{+}-K^{+}-ATPase activity were further monitored in the chilled comatose tilapia cultured in freshwater and various salinities (5 -20 ℃). The levels of blood hemoglobin, hematocirt, leucocrit and plasma lactate significantly decreased in comatose tilapia. Significantly higher levels of plasma glucose, cortisol and gill Na^{+}-K^{+}- ATPase activity were observed in comatose tilapia as compared with control fish (26 ℃) in all salinity groups. Higher levels of plasma chloride and osmolarity were only observed in the comatose tilapia than those in control fish (26 ℃) cultured at 20 salinity. Salt- acclimated tilapias were chilled to become comatose within a shorter period of time as compared with freshwater fish. Common carp is more to lerant to low temperature as compared to tilapia. Most of the biomedical parameters did not show the changes in common carp exposed to 8 ℃ . But, the levels of blood lactate, hematocrit, hemoglobin and leucocrit were consistently responsive to the stress of lower temperatures (24 ℃ to 4 ℃) in common carp.

九、已發表報告目錄：

1. Sun, L. T., Chen, G. R., and Chang, C. F. 1992. The physiological responses of tilapia exposed to low temperature. J. Therm. Biol., 17, 149-153.

2. Sun, L. T., Chen, G. R., and Chang, C. F. 1994. Characteristics of blood parameters and gill Na^{+}-K^{+}-ATPase in chilled comatose tilapia cultured in various salinities. Comp. Biochem. Physiol., 107A, 641-646.

3. Sun, L. T., Chen, G. R., and Chang, C. F. 1995. Acute responses of blood parameters and comatose effects in salt-acclimated tilapias exposed to low temperature. J. Therm. Biol., 20, 299-306.

4. Chen, G. R., Sun, L. T., Lee, Y. H., and Chang, C. F. 1995. Characteristics of blood in common carp, *Cyprinus carpio,* exposed to low temperatures. J. Applied Aquaculture, 5, 21-31.

一、計劃名稱：

中文：開發抗傳染性胰臟壞死病毒（IPNV）核糖酶轉殖魚類

英文：Development of ribozyme technology for the production of transgenic fish resistant to infectious pancreatic necrosis virus (IPNV)

二、執行期限：83年12月1日至86年6月30日共2.5年

三、執行單位：中央研究院動物所

四、美方合作單位及人員：

美國馬里蘭大學生物技術研究所　陳鐵雄

美國奧立崗州立大學微生物系　J. C. Leong

五、中方研究人員：吳金洌、徐亞莉、張繼堯

六、計劃目的：

以IPNV病毒(E1-S)作病毒病原，作cDNA選殖、定序及特性分析，設計與合成反意義寡核苷酸，並在CHSE-214細胞中測試其抗IPNV感染之功能，然後設計抗IPNV之核醣酶，並合成此核醣酶，及評估其在CHSE-214 細胞中之抗IPNV效益。最後將篩選出最有效益之核醣酶 minigene 作魚類基因轉殖，生產抗IPNV之核醣酶的轉殖魚類，並評估其抗病毒效益，以達開發抗感染性胰臟壞死病毒核醣酶的轉殖魚類。

七、執行成果摘要：

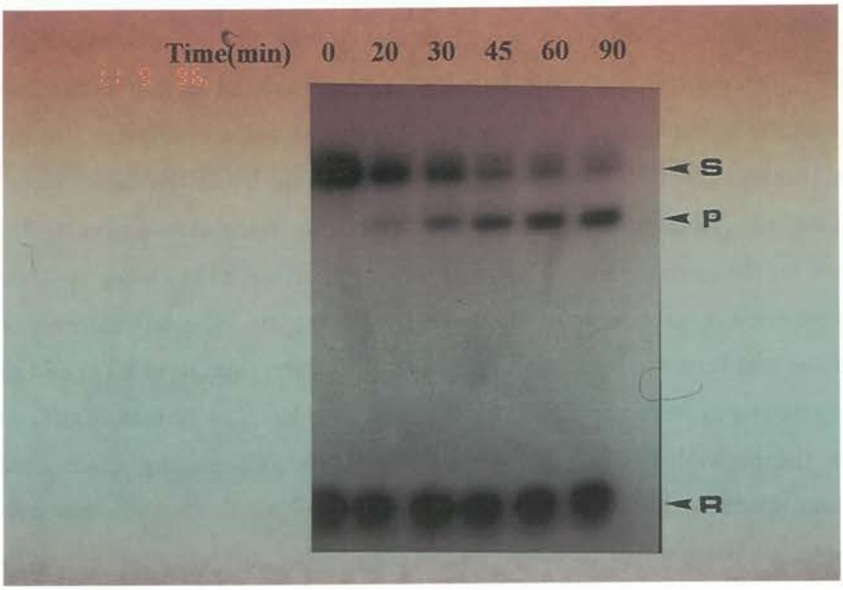
感染性胰臟壞死病毒（Infectious Pancreatic Necrosis Virus；IPNV）是一種高度傳染性的病毒，在台灣感染性胰臟壞死病毒，造成吳郭魚、鰻魚、虱目魚及文蛤等嚴重的損失。因此如何有效的防禦感染性胰臟壞死病毒是當前水產養殖界的重要課題之一。在本計畫中，設計出具有高度專一性的鎚頭型核醣酶（Hammerhead Ribozyme）及D型肝炎病毒核醣酶（Hepatitis D virus Ribozyme），期能順利切割感染性胰臟壞死病毒的核醣核酸。本實驗選用感染性胰臟壞死病毒的A段1346至1776 的基因片段，經由不同的核酸限制酵素的切割，產生出不同的線形的質體，利用Sp6聚合酶來進行活體外轉錄，合成出部份IPNV正股RNA，當做受質RNA；另一方面核醣酶的合成是直接利用含T7啟動子序列之去啟動核醣模版，進行活體外轉錄反應。結果發現，欲取得多量的RNA時，必先審慎評估T7啟動子下游之Sp6個核苷酸序列。在設計的14個核醣酶中，僅有RT-1與RT-3n兩個鎚頭型核醣核酸酵素能夠切割受質核醣核酸，此時的反應溫度為37℃及含12 mM的$MgCl_2$。同時為了要符合生物體內生理狀況則降低濃度的鎂離子的濃度由12 mM至6 mM，亦有不錯的反應效果。另一方面為了要提高切割標的RNA的反應成效，則嚐試著提高溫度，可使得原本於37℃無法進行切割反應的RT-7在50℃時可反應；然而適量地加添變性劑或PEG 6000亦可增加切割反應。

八、英文摘要：

Infectious pancreatic necrosis virus (IPNV) is contagious and widespread fish disease. In Taiwan aquaculture, IPNV has often caused heavy damage to the aquaculture industry for tilapia, eel, milkfish and clam. Development of effective control technology for IPNV infection has obviously become an important issue in fisheries development program and also imperative to continuing growth of culture industry. In this study, we designed highly specific hammerhead ribozyme and hepatitis delta virus ribozyme, synthesized the ribozyme RNAs and assayed the trans-cleavage activity of those ribozymes on the partial sense RNA of IPNV. Ribozyme RNAs were synthesized by T7 RNA polymerase transcription of synthetic DNA template. The downstream (+1 to +6) of T7 promoter was found to be a key factor for RNA production. Both RT-1 and RT-3n (hammerhead ribozyme) were capable to cleave substrate RNA in 12 mM $MgCl_2$ at 37℃. To match in the physiological environment of organism, decreasing the concentration of magnesium ion from 12 mM to 6 mM obtained good results. On the other hand, in order to elevate the efficiency of cleavage on the target RNA, increasing the reaction temperature and adding denaturants or PEG 6000 can improve the cleavage reaction. RT-7 was able to cleave substrate RNA in 12 mM $MgCl_2$ at 50℃, but that was not cut at 37℃.

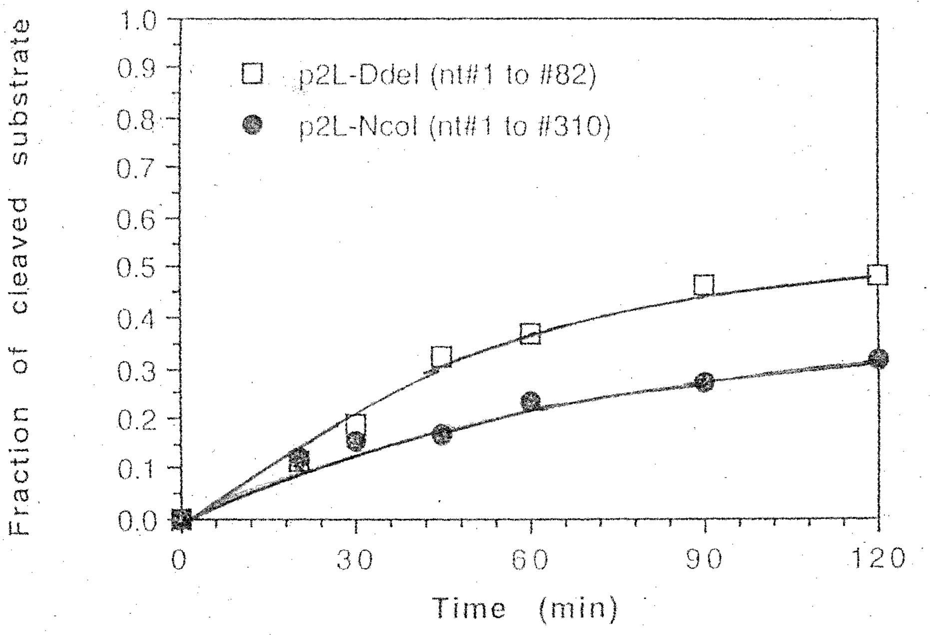
九、已發表報告目錄：

Wu, J. L., Long, S. S., Yih, S. W., Chang, C. Y., Liao, C. F., and Hsu, Y. 1993. Inhibition of Aquatic Birnavirus replication process in fish cells by viral antisense RNA. Animal cell technolog: basic and applied aspects. 6: 137-144.



圖一、RT-1/P2L-DdeI(nt# 1-82)雙分子系統之它割反應。
5nM受質RNA(S)、30nM核醣核酸酵素(R)，進行它割反應。兩溶液混合後，一起加熱95℃兩分鐘，回溫五分鐘後，於37℃下，以12 mM Mg^{2+}起始反應，於預設時間取出混合液，加入等體積之終止溶液，來終止反應。再以7M urea-10% PAGE進行電泳，分離結果。

Fig. 1. Efficiency of Cleavage the substrate P2L-Dde (nt# 1-82) by ribozyme RT-1. A react condition of ribozyme cleave the substrate as follwing: first, using both substrate (5nM P2L-Dde I, S) and ribozyme (30nM RT -1. R) to well mix, and denature in 95℃ for 2 min, renature in room temperature for 5 mins, then react in final concent ration of 12 mM Mg^{2+} at 37℃ for difference time. At the end of time point the reaction was stoped in stop solution, then analyzed the samples in 7M urea - 10% PAGE.



圖二、RT-1相對於不同受質RNA雙分子系統，於37℃下，進行它割反應。5nM受質RNA(P2L-DdeI或P2L-NcoI)，30nM核醣核酸酵素RT-1，進行它割反應。觀察切割反應速率與時間作圖。受質RNA與核醣核酸酵素反應之前一起heat denature，95℃兩分鐘，回溫五分鐘後，加入12mM的鎂離子，於37℃開始反應後，於預設時間取出混合液，加入等體積之終止溶液，進行電泳分離樣本，及分析結果。(□：表受質RNA為P2L-DdeI，●：表受質RNA為P2L-NcoI)。

Fig. 2. Comperation of cleavage efficiency of difference substrate by RT-1 ribozyme. The react condition as above, except add one substrate P2-NcoI, □: substrate 1, P2L-DdeI; ●: substrate 2, P2L-NcoI.

一、計劃名稱：

中文：草蝦桿狀病毒分子生物學及生物技術

英文：Molecular biology and biotechnology of monodon baculovirus(MBV)

二、執行期限：1989年7月1日至1993年6月30日共4年

三、執行單位：中央研究院動物研究所及國立屏東技術學院獸醫系

四、美方合作單位及人員：美國奧立岡州立大學微生物系 Dr. Joan C. Leong

五、中方研究人員：吳金冽、徐亞莉、董明澄

六、計劃目的：

建立草蝦桿狀病毒(MBV)之純化方法，萃取其DNA，並研究MBV之核酸及蛋白生化特性，建立MBV之培養系統，建立無特定病原養殖系統，研究MBV病原性，調查研究臺灣MBV疫病學。

七、執行成果摘要：

草蝦曾是台灣主要外銷之水產品。但在1988、1989年開始，台灣地區草蝦大量死亡，造成80％以上不能收成，經實驗證明是草蝦桿狀病毒(MBV) 之感染，加上環境緊迫所致。故急欲發展MBV之快速靈敏的診斷方法。首先建立純化病毒的方法，次利用抗昆蟲OpMNPV(Orgyia pseudotsugata MNPV)多角體蛋白抗體，作Western blot，來鑑定MBV多角體蛋白。SDS- PAGE分析蛋白大小。並作N端蛋白質定序分析。此外，抽取MBV之DNA，並根據七種昆蟲桿狀病毒之多角體蛋白基因及顆粒體蛋白基因之保守序列，設計並合成兩引子，利用PCR方法，擴增MBV多角體蛋白部分DNA，經定序，作為探針之用。由於多角體蛋白是在感染後期大量產生，因此利用DNA聚合酶的保守序列，設計並合成三套引子，及三個探針，經PCR 方法，只有一組PCR產生DNA擴增片段。且證明為DNA聚合酶片段，因病材短缺而中斷。

基於草蝦因病無法養殖，病材不易取得，而草蝦桿狀病毒對蝦類細胞專一性很強，若無法大量培養，許多研究無法進行。因此急欲建立草蝦淋巴組織（oka organ)之次級培養。首先從培養基的滲透壓、血清濃度、血清來源、pH來篩選基本培養基。其次測試營養添加物，如碳水化合物、胺基酸、維他命C、Buffalo rat liver (BRL)條件培養基、和硒。但都無法繼續培養，因此針對生長因子作添加。終於在添加鹼性纖維細胞生長因子(bFGF)20ng/ml(F-20)下，細胞可繼代培養，現已達150代以上。即利用L-15 + 5g/l NaCl + 1g/l glucose + 10%FBS + 20ng/ml bFGF + antibotics。今已完成細胞株(PMO)的特性分析、生長特性、Isozyme pattern、染色體數目、以及病毒感受性等。

自1989年7月至1993年6月（四年），在台灣南部共採集草蝦苗(DL15-PL-35) 830例（50-80尾／例），進行MBV流行病學的研究調查，及應用伊紅(Eosin)及孔雀綠(Malachite green)染色快速診斷，組織病理學檢查及電顯檢查，確實診斷MBV感染，同時

亦進行一般蝦病病性鑑定。結果1989～1993年蝦苗之MBV感染率，分別為85.3%, 87.6%, 79.5%及80.5%，平均83.2%；養成池草蝦MBV感染率，分別為46.0%, 47.1%, 49.8% 及50.5%，平均48.4%。

八、英文摘要：

Tiger shrimp was one big item of export aquaculcure in Taiwan. Since 1988, 1989, the mass mortality of tiger shrimp occurred in this area, about 80% of farms could not harvest. From the experimental evidence, this was caused by monodon baculovirus(MBV), combined with environmental stress. Therefore, the study of molecular biology and biotechnology of monodon baculovirus is necessary. First, the purification of MBV was set up. Then, the polyhedrin polyclonal antibodies against insect OpMNPV(Origyia pseudotsugata MNPV) was used to identify the polyhedrin of MBV. The molecular size of viral proteins was analyzed by SDS-PAGE. Amino acid sequece of N-terminal of MBV polyhedrin was determined. Based on the conserved sequences of polyhedrin and granulin genes of 7 insect baculovirus, two primers was designed and synthesized, and the extracted DNA of MBV, the amplified partial polyhedrin DNA was synthesized by polymerase chain reaction. The amplified DNA was ligated with plasmid vector, sceened and sequenced. This segment could be used as probe. Since polyhedrin was synthesized at the late stage of infection, the DNA polymerase of MBV was chosen as diagnostic target due to its early expression after infection. 3 set primers and 3 probes were designed and synthesized. Only one PCR-DNA product was amplified. The further experiments were stopped due to short of illed shrimp.

Based on tiger shrimp could not be able to culture. the illed sample was hard to be obtained. Many studies of MBV were blocked due to the high species specificity of MBV. The establishment of subculture system of Peneaus monodon of oka organ is urgent. The basic culture conditions including culture medium, pH, temperature, sources, concentration and osmolality of serum. Then, supplement of specially-defined nutrients including carbohydrates, amino acids, vitamins, and some conditional media were screened. The nutrient medium could support the cell to grow, not for the cell subculture. Finally, growth factor was also to be defined. Cell treated with basic fibroblast growth factor (bFGF) 20ng/ml(F-20) could be subcultured for more than 150 passages. However, the final culture medium is L-15 + 5 g/l NaCl + 10% FBS + antibiotics. The characterization of the cell line PMO, growth condition, isozyme pattern, chromosome number and virus susceptibility were completed recently.

In order to understand the epizootiological status of monodon baculovirus (MBV)

infection of tiger shrimp in southern Taiwan. A total of 830 cases (50-80 samples/case) of postlarva from postlarval stocking ponds and 201 cases (10-15 samples/case) of juvenile to adult from growout ponds were collected for the survey in the period of July 1989 to Jun 1993. A combination of the rapid eosin staining method with histopathological study and electron microscopy was employed for detection rates in 1989 to 1993 for postlarval stocking ponds were 85.3%, 87.6%,' 79.5% and 80.5% respectively with the average of 80.5%, however those of the growout ponds were 46.0%, 47.1%, 49.8% and 50.5% respectively with the average of 48.4%.

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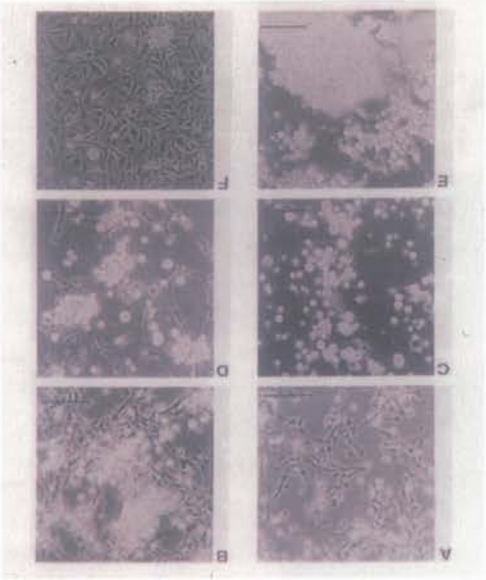

Fig. 1. Effects of basic fibroblast growth factor (bFGF) on morphology of the studied shrimp cell cultures. (A) Control after 2 days incubation, (B) 20ngml^{-1} bFGF - treated cells after 2 days incubation. (C) bFGF(F-20)-treated cells after 11 days incubation, (D) F-20-treated cells after three passages with the feeder layer cells, (E) F-20 treated cell after 39 passages, (F) F-20-treated cells after 60 passages. Bar is 75 μ m.

M	F	G	D	N	M	M	D	D	V	E	H	L	S	G
ATG	TTT	GGT	GAT	AAT	ATG	ATG	GAT	GAT	GTT	GAA	CAT	TTA	TCT	GGT
	C	C	C	C			C	C	C	G	C	G	C	C
		A							A			CTT	A	A
		G							G			C	G	G
												A	AGT	
												G	C	

Fig. 2. N-terminal Aminco acid sequence and its DNA sequence of the polyhedrin (56kd) of MBV.

Fig. 3. Identification of the polyhedrin cloue of MBV.

(A) The PCR product of 0.5Kb DNA fragment of MBV was identified by Southern blot of OpMNPV polyhedrin probe. Then the 0.5Kb DNA was cut out, and logated with pGEM7Z f(-), and transformed into *E. coli.* Then the plasmid was purified and analyzed by 1% agarose gel.

(B) Southern blot of (A) with the probe of OpMNPV pelyhdrin. Lane 1 and 2: Eco RI cut DNA of Different plasmid of MBV polyhedrin clones, lane 3: λ /Hind Ⅲ marker, 3.0Kb: pGEM 7Z f(-), 0.5Kb:insert DNA.

一、計畫名稱：

中文：養殖青魚死亡原因之研究

英文：Studies on the causes of mortality of cultured black carp, *Mylopharyngodon piceus*

二、執行期限：1989年07月01日至1992年06月30日，共3年。

三、執行單位：台灣省水產試驗所

四、美方合作單位及人員：美國農部

五、中方研究人員：廖一久、劉富光

六、計畫目的：

青魚為中國大陸最重要的淡水養殖魚類之一，不僅味美且成長迅速，甚具經濟價值。在本省俗稱黑鰡，由於體型碩大，拉勁十足，遂成為本省淡水遊釣場的最愛。由於本省青魚繁養殖的相關資料尚付闕如，有鑑於此，本試驗乃在探討青魚的繁養殖技術以及養殖上必須注意的環境因子，同時究明青魚的營養需求，藉以建立養殖基礎資料，俾供養殖業者參考，從而提升養殖經濟效益。

七、執行成果摘要：

在青魚的繁殖季節(每年約在4-7月)，挑選10年齡，體重約18-30Kg 的成熟雌魚，經激素打針催熟後，在水溫24-28℃情況下，受精卵24小時左右可孵化。魚苗以餌料生物及鰻粉飼料飼育3個月，成長至13.22±4.22g。另以每尾平均體重100g的青魚做密度試驗的結果發現，低密度組成長率、飼料效率(分別為38.0%及67.6%)均比高密度組佳(分別為22.5%及45.4 %)。如以體重650g的魚體進行試驗，亦有類似之結果。

關於青魚幼苗最適蛋白質需求量之探討，以酪蛋白為主要成分調製不同蛋白質含量(20-45%)之多組配方中，顯示蛋白質含量在30%以上時，其成長優於低蛋白質者(含36.06%時，可達最大成長率)。由青魚對數種水溶性維生素需要性試驗得知，青魚在缺乏維生素B_6時，其成長率最低，且死亡率高達46.67%，其次為維生素B_2與C缺乏組(13.33%)。

在環境適應力方面，不同大小的青魚，在水溫10-35℃範圍下，耗氧量為0.9-9.2 mg/min/Kg；致死溶氧量則為0.29-2.34mg/l。而致死高溫為36-37 ℃，致死低溫為6.5 ℃，致死鹽度則在15-18℃之間。另由試驗測知，氨-氮(Amonia-N)對青魚的96TLm約10ppm亞硝酸-氮(NO_2-N)則為80ppm。顯然，氨-氮對青魚的毒性遠大於亞硝酸-氮。

八、英文摘要：

As the black carp (*Mylopharyngodon piceus*) is a fast growth and tasty freshwater fish, it is considered to be an economically important cultured spceies in China. In addition, due to its big size, it has been also gaining more and more interest as recreational fish in Taiwan. The major purpose of the studies was to investigate the techniques for

culture and induced ovulation of black carp. Moreover, the nutritional requirements and the tolerance to environmental parameters were also determined in order to establish useful datas for the reference of culturists.

During the spawning season (from April to July) of black carp, mature broodstock weighed from 18-30kg at an average of 10 age were selected to induce successfully stripped ovulation and spontaneous spawning. At a temperature of 24-28℃, hatching time was around 24h after fertilization. The larvae wre fed live food organisms and commercial formulated feed. They had 13.22 ± 4.22g body weight after 3-month rearing period.

Study was conducted to investigate the optimal protein requirement of black carp. Experimental diets containing casein as the primary protein source were grouped into 6 protein levels (from 20 to 45%). The weight gain of fish fed higher than 30% protein was significantly higher than those fed other level of dietary protein (maximun growth was found in diet with 36.06% protein level.)

Experiment was also carried out to determine various water-soluble vitamin requirement of black carp. Fish fed with pyridoxine-free diet resulted in slowest growth and highest mortality (46.67%). Higher mortality (13.33%) were also found with groups of riboflavin-free and ascorbic acid-free diet.

The tolerence of the black carp towards various environmental parameters was tested. The oxygen consumption of two different fish sizes (average body weight 10.5g-12g and 4.0g-4.5g) was in the range of 54-552mg/kg/hr under water temperature ranging from 10 to 35℃. The lethal dissolved oxygen content was estimated as 0.28-2.34mg/l. In the study of the tolerable temperature range of black carp, it was also found that all fish survived between 7-35℃, but fish began to die at 6.5℃ and 36℃

中美農業科學合作研究計畫執行結果報告

Final and Progress Reports of COA-USDA Agricultural Science Cooperative Projects

牧業領域 (Livestock)

一、計畫名稱：

中文：本省家禽流行性感冒之監測

英文：Avian influenza surveillance in Taiwan. R.O.C.

二、執行期限：1986年7月1日至1993年6月30日，共6年。

三、執行單位：國立中興大學獸醫研究所

四、美方合作單位及人員：

USDA-ARS-South Atlantic Area

Charles W. Beard

五、中方研究人員：謝快樂、呂榮修、李永林、陳志豪、黃文正、邱新育。

六、計畫目的：

家禽流行性感冒的禽類繁多，病毒本身遺傳基因相當特別，以致經常有新的病毒株之出現，由病毒分離和抗體調查，知道本病遍布全省各地，而所分離病毒株血清亞型逐年改變中，所幸尚無類似所謂雞瘟的強毒株之出現，由以往本病全世界的流行記錄含H5、H7的毒株均曾引起家禽大量死亡，本計畫擬對本病毒監測及研究改進診斷方法。

七、執行成果摘要：

由以往五年多之監測，AIV遍佈全省已成不爭的事實，且每年均有新的血清亞型出現，對所分離的AIV進行致病力之實驗也證實雖然沒有強毒型AIV出現，但具有中間毒力的毒株則在多個種雞場出現，有些雞場抗體陽性率更高達90%以上。

由二個種雞群每月採血共採645支血清，以免疫擴散法測定，結果AI抗體陽性率為54.73%，陽性血清再以α-procedure之HI test得知抗體的血清亞型為H1，H2，H6，H8和H9。在冬季(10-12)由18個種雞場採取3415支血清以免疫擴散法測定，結果AI抗體陽性率為34.73%。

建立融合瘤以期產生對抗H5亞型之單源抗體，經過二次單細胞挑選，篩選到三株具有高產生抗體能力之融合瘤，分別以培養皿培養及注射到BALB/c小白鼠來產生單源抗體，此抗體以HI test力價達≧256，唯可對抗血清亞型H5，H6，和H8，但對其他亞型則為全為陰性，以間接螢光抗體染色，結果與HI test有類似情況亦即可以在H5，H6和H8亞型病毒感染之細胞出現螢光，而在其他亞型者則無螢光出現。

本單源抗體其isotype為IgG1，其輕鏈屬於Kappa chain。此單源抗體與SDS-PAGE之病毒蛋白在西方轉印酵素標示反應中無法出現反應之線條。

各AIV亞型亦以家兔製造高免血清，唯一亞型之抗體可與多種亞型之病毒在HI test中呈交叉反應之情形相當普遍，製造單一亞型之特異抗體有待繼續努力。

八、英文摘要：

Sera from chicken breeding farms were collected for routine AI antibody surveillance by means of AGP and HI tests. Monthly monitored results of two flocks indicated that the AI antibody was positive all year around. The subtypes of the antibody were H1, H2, H6, H8, H9 when the positive sera were tested by means of HI tests in α-procedure against the subtypes H1 through H12. A total serum samples of 643 from the two flocks had an average positive rate 54.73%, while a total of 3415 samples from 18 breeding farms collected in winter season had average positive rate 34.73%.

Another effort was made to establish a hybridoma to produce monoclonal antibody against AIV subtype H5 Three clones of hybridoma with strong antibodyproducing ability were established after two single-cell picking. The monoclonal antibody was produced both in cell culture and in BALB/C mice, then subjected to HI tests, IFA test, westernblotting against SDS-PAGE AI viral protein, and immunoglobulin isotype determination. Although it's a monoclonal antibody the antibody level was $\geqq$256 in both HI and IFA (indirect fluorescent antibody) tests against AIV subtypes H5, H6 and H8, while negative against all the other subtypes. The antibody did not reacted with the denatured SDS -PAGE viral protein in western-blotting. The isotype of the antibody was IgG1 with kappa light chain.

Hyperimmune sera against all the AIV subtypes were also prepared in rabbits. However, the phenomenon that serum prepared against one subtype could react with several subtypes was also noted.

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一、計畫名稱：

中文：台灣豬假性狂犬病之流行病學及預防。

英文：Epizootiological survey and prevention of pseudorabies infection in Taiwan.

二、執行期限：1986年12月10日至 1991年11月30日，共五年。

三、執行單位：國立中興大學獸醫學系

四、美方合作單位及人員：USDA, Dr. William L. Mengeling

五、中方研究人員：王吉德、張天傑、杜文珍、費昌勇、何維莊

六、計畫目的：瞭解台灣豬假性狂犬病之流行病學類型並研發一種有效的防治方法。

七、執行成果摘要：

在流行病學方面，本計畫連續對屠宰場豬隻調查抗體陽性率，發現自民國七十六年起的62.8%漸增至民國八十一年的73.2%，單月的陽性率甚至曾在七十九年四月高達94.2%。顯示本省目前豬假性狂犬病之感染相當嚴重。本計畫並比較了全省各分離株之DNA限制脢圖分析，發現各毒株間都有差異，顯現成地方性流行之現象。但本省毒株與美國毒株之間則有顯著之差異。

在診斷方法上，我們開發了假性狂犬病毒的單源抗體，可作為疾病診斷及研究之工具。也建立了酵素結合免疫吸附法 (ELISA) 可用以作大量血清檢體的快速檢測。並以免疫過氧化脢檢測法檢測病毒在感染豬體內之分布，發現病毒主要存在於淋巴球或巨噬細胞內。最後並建立了一種聚合脢連鎖反應法 (PCR)，可用來檢測環境中或感染豬排泄物內是否含有病毒。其敏感性高達0.1個感染單位，是目前所知之檢測方法中最敏感的。

在預防方法之研究上，我們比較了四種市售疫苗的免疫效力，發現四種之間並無明顯差異。但若母豬已有免疫，則會影響仔豬疫苗接種之免疫效力。母豬的移行抗體平均半衰期約為20.3天。本計畫並研究建立了缺胸腺核甘激脢病毒株之誘導、篩選、及確認的方法，可作為日後開發基因缺損活毒疫苗的基礎。

八、英文摘要：

Pseudorabies (PR) in Taiwan has already become a serious problem in swine industry. Sporadic outbreaks of PR among the PRV persistently infected herds were observed. It causes severe losses in neonates as well as in pregnant sows. Early study indicated, when a herd infected with a PR virus (PRV), the virus would remain in the herd and cause latent infections in pigs. Therefore, it is very difficult to clean up the virus from the PRV -contaminated farms. This project was proposed to investigate the epizootiological patterns of PRV infection in Taiwan and attempted to develope an effective measurement to control the PRV infection.

This report investigated PR prevalence in Taiwan in finishing pigs. The survey of

PR antibody in 20,341 serum samples collected from three slaughter houses from 1987 to 1991, a five year period, indicated that the prevalence of PR in Taiwan is very high. The prevalence of PR antibody was 62.8% in 1987, 64.3% in 1988, 73.0% in 1989, and 73.8% in 1990. However, the prevalence came down to 65.5% in 1991. Northern Taiwan generally shows a higher prevalence than the central and the southern regions of Taiwan. This report also indicated that spring usually had a higher PR prevalence within the period investigated.

The method of polymerase chain reaction (PCR) has been adapted for the detection of genetic sequences of the pseudorabies virus (PRV). Three primer pairs of sequences were chosen and their specificities on the detection of PRV genetic sequences were compared. Nine strains of PRV and the Cooper strain of bovine herpesvirus 1 (BHV-1) were used for the comparison of the specificity of these primer pairs on the amplification reaction. The primer pair selected from the BamHI-15 fragment not only produced an amplified product of 283 base pairs (bp) when reacted with the PRV strains, but also generated a product of about 194 bp on the reaction with the BHV-1 DNA. The reaction using the primer pair chosen from the immediate early gene only generated a specific PCR product of 271 bp for the UCD and SHOPE strains, but not for any of the Taiwan strains. When the primer pair sequences adapted from the gX glycoprotein gene were used, a specific DNA product of 294 bp was generated from the enzymatic amplification of all the PRV strains tested, but not of the BHV-1 or cell DNA. The sensitivity of the PCR system using gX gene as the primer pair could be as high as 1 PFU of PRV or 0.1 pg of PRV DNA by ethidium bromide staining, and 0.1 PFU by Southern blot hybridization. We also establish a method for induction, selection, and identification of a thymidine kinase deficient mutant. It will become the basis of development of a gene-deleted mutant.

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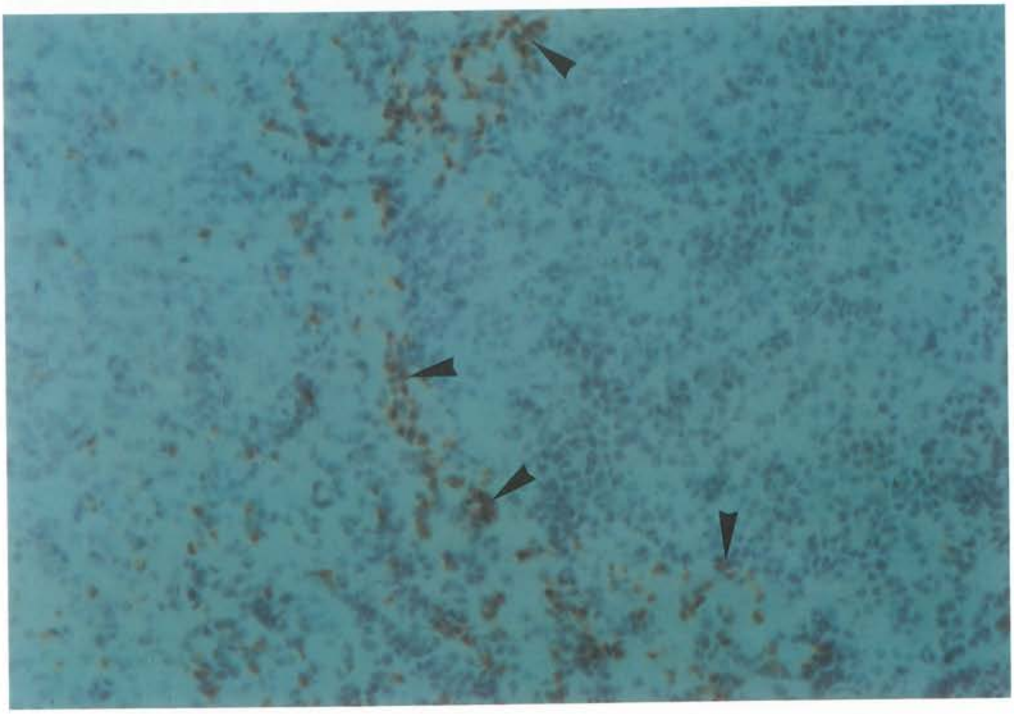
8. Chung, Y. T., W. J. Du and T. J. Chang. 1995. Restriction endonuclease analysis of the genomes of pseudorabies virus strains isolated in Taiwan. Taiwan Jour. Vet. Med. & Anim. Husb., 65:11-20.

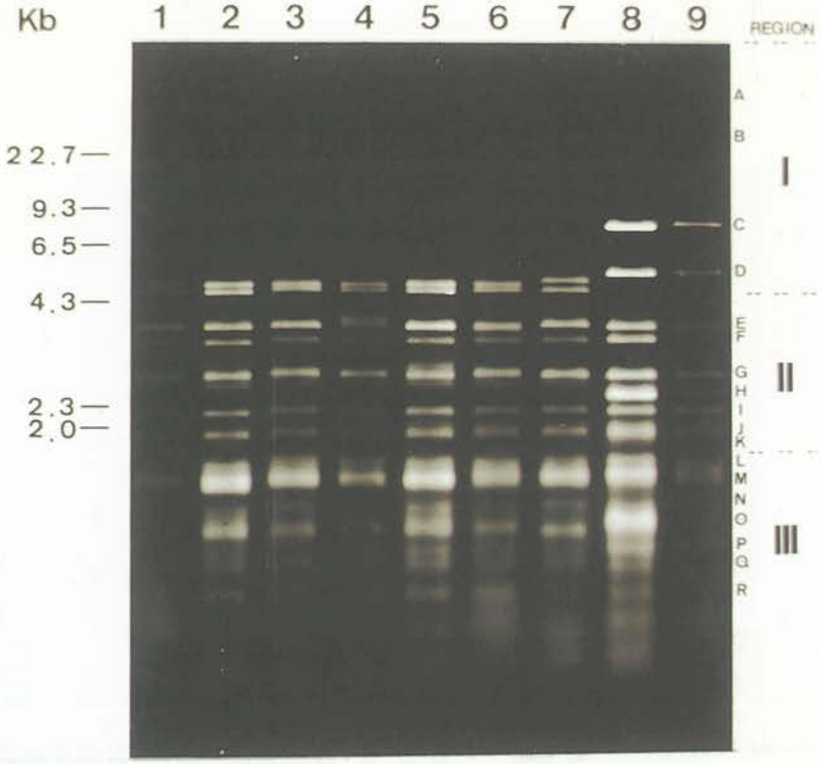


圖一、以免疫過氧化酶染色法檢測PRV感染豬之淋巴結的冷凍切片結果(放大400倍)。箭頭所指處乃副皮質陽性反應區。

Fig. 1. The results of immunoperoxidase test on the cryostat section of the lymph node of PRV-infected pig in 400 folds magnified. The arrows show the positive reactors in the paracortex area.

圖二、以TNL株*Bam* HI第13切割片段與各PRV分離株DNA的南方雜交試驗結果。各PRV分離株DNA的*Bam* HI切割片段經電泳分析、轉移至尼龍紙上後，以標識有32P的TNL株*Bam* HI第13切割片段進行南方雜交試驗結果。C行是TNL株DNA 的*Bam* HI切割片段電泳結果。第1行至第9行是南方雜交試驗結果。第1行TNL株，第2行TP1株，第3行P3株，第4行CS株，第5行NCHU株，第6行CN株，第7行PT 株，第8行UCD株，第9行Shope株。

Fig. 2. Hybridization of DNA of PRV isolates by *Bam* HI fragment 13 of TNL isolate DNA. The DNA of different isolates were digested by *Bam* HI, electrophoresed, transferred to a nylon paper, and hybridized by a *Bam* HI fragment 13 of PRV TNL DNA labeled with ^{32}P. Lanes C and 1 were loaded with cleaved DNA from isolates of TNL; lane 2 from TP1; lane 3 from P3; lane 4 from CS; lane 5 from NCHU; lane 6 from CN; lane 7 from PT; lane 8 from UCD; lane 9 from Shop.



圖三、不同分離株假性狂犬病毒基因體DNA經限制酶*Sma* I切割後，以0.7%瓊脂膠體電泳分析之結果。圖上方1至9行分別為：第1行TP1株，第2行P3株，第3行CS株，第4行NCHU株，第5行CN株，第6行TNL株，第7行PT株，第8行UCD株，第9行Shope株。

Fig. 3. Electrophoretic patterns of PRV DNA digested with *Sma* I. Lane 1 was loaded with digested DNA isolated from strain TP1; lane 2 from P3; lane 3 from CS; lane 4 from NCHU; lane 5 from CN; lane 6 from TNL; lane 7 from PT; lane 8 from UCD; lane 9 from Shope.

一、計劃名稱：

中文：豬附紅血球立克次氏體病之研究－診斷、免疫學與防治

英文：Eperythrozoonosis in swine－diagnosis, immunology and control

二、執行期限：1987年9月1日至1990年9月30日，共三年

三、執行單位：台糖公司畜產研究所

四、美方合作單位及人員：

Department of Veterinary Pathobiology, College of Veterinary Medicine
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Drs. A.R. Smith and J.F. Zachary

五、中方研究人員：徐興鎔

六、計畫目的：探討豬附紅血球立克次氏體之診斷方法、免疫學與防治方法

七、執行成果摘要：

(一)開發血清學技術包括酵素連結免疫吸附分析法 (ELISA)，以篩檢豬附紅血球立克次氏體病 (EP) 感染豬隻－由試驗結果證實ELISA檢驗法的特異性與靈敏度極高，又經濟、快速、操作方法，可作為全省大量豬隻篩檢之用。本研究成果業已在1992年美國獸醫學會雜誌中刊載。

(二)應用ELISA調查本省豬隻中EP之感染情形，發現本省豬群感染率在15.1之間。

(三)開發本病之預防控制與有效治療方法，該方法經台糖公司以及本省其他養豬場應用以來，效果顯著，目前業已廣泛推廣應用於全省養豬場。

(四)完成豬附紅血球立克次氏體病之流行病學調查。

(五)完成豬附紅血球立克次氏體病與豬沙氏桿菌症合併感染之研究，此研究成果可作為豬*E. suis*與沙氏桿菌病防治之重要參考資料。

(六)完成豬附紅血球立克次氏體病之免疫與血液反應之研究。

八、英文摘要：

The works accomplished include:

1. An enzyme-linked immunosorbent assay (ELISA) has been developed to detect antibodies to *E. suis* in porcine sera. The results showed that the ELISA is reliable and more sensitive than the IHA test. ELISA should be a valuable aid in experimental and epidemiological studies and differential diagnosis.
2. An islandwide survey for the prevalence of *E. suis* infection in swine in Taiwan has been completed. The survey has provided up-to-date information as a basis for the control of EP. The prevalence of infection ranged from 15.1 to 80.50%.
3. Two programs have been developed for the control of EP in naturally occurring swine herds. The first program involving use of arsenicals, antibiotics and certain manage-

ment procedures has substantially reduced death and prevented E. suis infection. However, the other one, the combination of oxytetracycline injection and feed medication, may reduce clinical signs and increased weight gains, but it was not able to eliminate *E. suis* from the blood. Both control programs or methods have been wildly applied here in Taiwan, and a good response has been obtained so far.

4. The relationships between *E. suis* and *S. choleraesuis* in swine has been studied. The data is valuable for the control of *E. suis* and *S. choleraesuis* infections.
5. The immunologic and hematologic responses in intact and splenectomized pigs with experimentally induced *E. Suis* infection has been investigated.

九、已發表報告目錄：

Hsu, F. S., M. C. Liu, S. M. Chou, J. F. Zachary and A. R. Smith. 1992. Evaluation of an enzyme-linked immunosorbent assay and a modified indirect hemagglutination inhibition test for detection of *Eperythrozoon suis* antibody in swine. Am J Vet Res, Vol 53. 3: 352 -354.

一、計劃名稱：

中文：牛邊蟲病流行病學、血清學與防治之研究

英文：Epidemiology, serology and control of bovine anaplasmosis in Taiwan

二、執行期限：1990年7月1日至1994年9月30日，共四年

三、執行單位：台灣省立家畜衛生試驗所、台灣糖業公司畜產研究所

四、美方合作單位與人員：

Department of Vet. Pathobiology, College of Vet. Med.,
University of Illinois, USA
Dr. L. Hungerford

五、中方研究人員：蘇杰夫、徐興鎔

六、計劃目的：

(一)分離牛邊蟲病之病原 (牛邊蟲 *Anaplasma marginale*)

(二)開發血清學診斷方法

(三)調查本省反芻動物牛邊蟲病之感染率

(四)開發牛邊蟲疫苗

七、執行成果摘要 (中文)

(一)本研究自台灣地區牛群中成功地分離到牛邊蟲病之病原“牛邊蟲，*A. marginale* (Ana)”

(二)開發三種血清學診斷技術包括補體結合反應 (CF)、間接螢光抗體法(IFA) 與間接酵素結合免疫吸附法 (ELISA)。

(三)應用CF法調查台灣地區牛隻邊蟲病之感染情形，其結果如下：

1.應檢台北等8縣市牛群，結果牛邊蟲之陽性 (感染) 率在2.7至62.3% (平均為30.9%)。

2.所有8個受檢縣牛隻均見牛邊蟲之污染，其中以台北縣為最高(62.3%)，新竹縣(47.5%) 與苗栗縣 (35.8%) 次之，其他依次為台中縣 (16%)、屏東縣(12.5%)、台南縣 (11.8%) 與台東縣 (2.7%)。詳中華獸醫學會雜誌

3.另調查台北、桃園、屏東與台東等4縣之羊隻500頭，結果陽性縣有台北 (7%) 與桃園 (4%)。

(四)開發牛邊蟲 (*A. marginale*) 死毒與減毒疫苗 (MLAm)，證實MLAm對牛邊蟲之防疫效果較死毒疫苗為佳。

(五)本研究另自本省牛群中分離到*A. centrale*，此病原經減毒製成疫苗可防治牛邊蟲病。

八、英文摘要：

The works accomplished include：

1. *A. marginale,* an infectious agent, was successfully isolated from naturally infected cattle originating from a farm in northern Taiwan.
2. Three serological tests, including complement-fixation test (CF), indirect fluorescent test (IFA) and enzymelinked immunosorbent assay (ELISA) were developed to detect antibodies to *A. marginale* in ruminants.
3. Comparative testing among the 3 serological tests showed that the CF had the highest sensitivity.
4. Of the 915 cattle serum samples from 8 prefectures tested, 283 (30.9%) were positive. Collectively, all 8 prefectures had *A. marginale* antibodies. Among these, Taipei prefecture had the highest prevalence (62.3%), followed by Hsinchu (47.5%), Taoyuen (43.2%), Miaoli (35.8%), Taichung (16%), Pingtung (12.5%), Tainan (11.8%) and Taitung (2.7%). In goats only two of the 4 prefectures submitting serum samples had *A. marginale* antibodies with prevalence of 7 and 4%, respectively. (Ref. to J. Chin Soc Vet Sci 19(2): 79
5. An inativated and two modified live *A. marginale* immunogens (MLAm) using Taiwan isolate were developed and have been undergoing a laboratory trial to evaluate their safety and efficacy. The results indicated that MLAm has the potential for against bovine anaplasmosis.
6. *Anaplasma centrale* was successfully isolated from naturally infected cattle in northern Taiwan and has been attenuated to be a vaccine against bovine anaplasmosis.

九、已發表之報告目錄：

1. Su, J. F., and F. S. Hsu. 1993. Serolegical assays and seroprevalence of ruminant *Anapasma marginale* infection in Taiwan. Chin Soc Vet Sci 19(2): 79-89.

一、計畫名稱：

中文：傳染性鼻氣管炎自然感染牛與免疫牛鑑別診斷之研究

英文：Differentiation of natural infection from vaccination with inactivated virus of infectious bovine rhinotracheitis in cattle

二、執行期限：1988年7月1日至 1992年6月30日，共四年。

三、執行單位：國立中興大學獸醫學系

四、美方合作單位及人員：

University of California, Davis,

Dr. Yuan Chung Zee

五、中方研究人員：吳福明、張天傑、徐慶霖、宣詩玲

六、計畫目的：

1. 開發牛傳染性鼻氣管炎病毒之單源抗體以作為快速診斷試劑。
2. 建立一種酵素免疫吸附法以檢測抗體力價。
3. 利用病毒封套蛋白作為抗原以研發一種次單位疫苗。
4. 研發一種鑑別牛傳染性鼻氣管炎病毒感染牛與免疫牛的方法。

七、執行成果摘要：

牛傳染性鼻氣管炎病毒（Infectious bovine rhinotracheitis virus; IBRV）是引起牛隻傳染性鼻氣管炎的病因，已廣泛污染台灣多數酪農場。本病有高度的感染率，以呼吸症狀為主，造成產乳產肉率下降，且對懷孕母牛造成流產，痊癒牛隻易形成潛伏性感染（Latent infection），而成為本病之帶原者（virus-carrier）。帶毒牛偶而傳染病毒給具有感受性的其他牛隻，而造成酪農莫大的損失。本試驗研發了二十株單源抗體，可作為疾病診斷及研究之工具。我們亦比較了感染牛與疫苗接種牛的血清與病毒蛋白的作用情形。一般而言，疫苗接種牛的血清中的抗體可與較多的病毒蛋白作用，但其結果不一定。為了尋求快速而敏感的診斷方法，本實驗利用聚合酶連鎖反應（Polymerase chain reaction；PCR）以偵測牛傳染性鼻氣管炎病毒核酸。本實驗利用牛傳染性鼻氣管炎病毒醣蛋白gⅣ 基因的核苷酸序列來設計此段DNA 片段兩端的引子（primers），經聚合酶連鎖反應可偵測八株 IBRV，但不與假性狂犬病毒（Pseudorabies virus；PRV）DNA 反應，並且以此套引子進行敏感性試驗，結果顯示聚合錨連鎖反應偵測病毒核酸之敏感性可達0.01 PFU 感染單位之病毒及0.01pg的病毒核酸。因此，由本實驗知，選自gIV基因的引子進行聚合錨連鎖反應可得高度特異性、敏感性及快速診斷牛傳染性鼻氣管炎病毒核酸。

八、英文摘要：

Infectious bovine rhinotracheitis (IBR) is a respiratory disease of cattle characterized as an acute, contagious, febrile infection. The etiologic agent, IBR virus, is now known

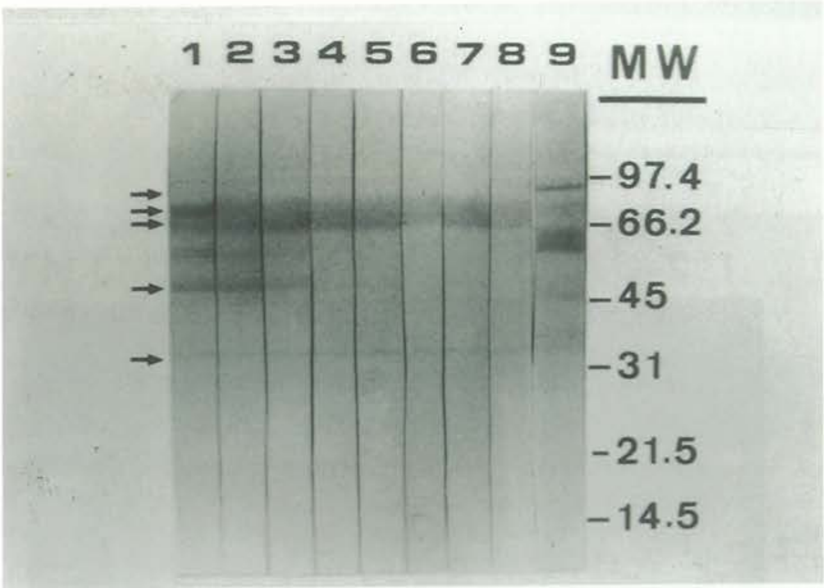
as bovine herpesvirus 1 (BHV-1) is responsible for a variety of clinical manifestations including rhinitis, tracheitis, and encephalitis. The goal of this project is to develop a method to differentiate the infected carriers from vaccinated cattle. In this report, we had (1) developed and selected twenty hybridoma clones secreting monoclonal antibodies (Mab) reacted with IBRV proteins, and most of the Mabs reacted with 70K and 77K proteins; (2) compared the restriction endonuclease digestion patterns of six Taiwan isolates of IBRV, and the PT and KS3 isolates had obvious variations; (3) compared the patterns of polypeptides precipitated by sera from IBRV- infected cattle and sera from vaccinated cattle, and it showed that the patterns were similar. The sera from vaccinated cattle generally precipitated more polypeptides than the sera from IBRV- infected cattle, but the results were not consistent for all tested serum samples; (4) established the ELISA assay for detecting antibody titer of IBRV using the purified virus or nucleocapsid as an antigen, and we found the ELISA titer had a better correlation with serum neutralization titer when using the purified virus as the coating antigen; (5) investigated the immune responses induced by the polypeptides in inactivated vaccine, and we found that the inactivated vaccine contains epitopes of early proteins which would also generate good immune responses; (6) devised a sensitive and specific method using nested double polymerase chain reaction(PCR) to detect the genetic sequences of the IBRV. The sensitivity of this nested double PCR system could be as high as 0.01 plaque forming unit of IBRV or 0.01 picogram of IBRV DNA. It indicated that the nested double PCR method could be used for rapid detection of IBRV in clinical specimens from cattle.

九、已發表報告目錄：

1. Chung, Y. T., T. Y. Chang, T. J. Chang, and F. Wu. 1993. Detection of infectious bovine rhinotracheitis virus DNA by the nested double polymerase chain reaction method. J. Chin. Soc. Vet. Sci. 19, 26-37.

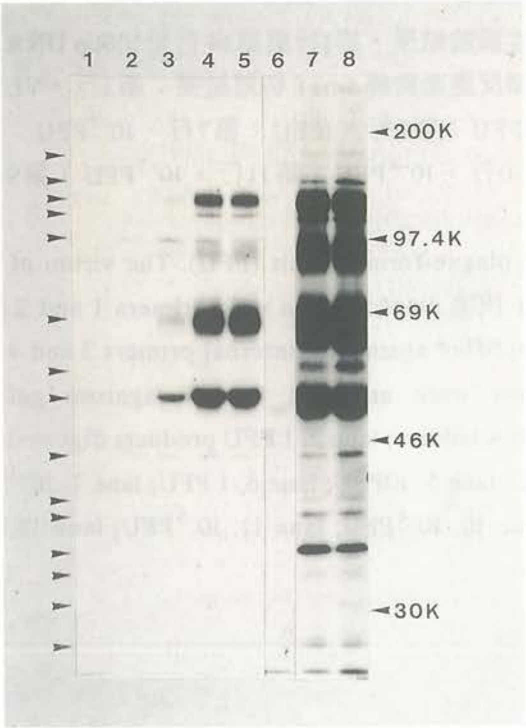
圖一、IBRV引子3,4的巢氏聚合酶連鎖反應敏感性試驗結果。第1行與第14 行是100bp DNA標記；第2行YL株1 PFU的巢氏聚合酶連鎖反應產物經*Sma* I 切割結果；第3行，YL株10^3PFU；第4行，10^2PFU；第5行，10PFU；第6行，1PFU；第7行，10^{-1}PFU；第8行，10^{-2}PFU；第9行，10^{-3}PFU；第10行，10^{-4}PFU；第11行，10^{-5}PFU；第9行，10^{-6}PFU；第9行，10^{-7}PFU。

Fig. 1. Sensitivity of the PCR assay based on the plaque-forming unit (PFU). The virion of IBRV strain YL was used as the target for PCR amplification with primers 1 and 2. Portions of the first PCR products were amplified again with internal primers 3 and 4 for nested PCR. The amplified products were analyzed by 2% agarose gel electrophoresis. Lane 1 and 14 are 100 bp DNA ladders; lane 2, 1 PFU products digested with *Sma* I; lane 3, 10^3PFU; lane 4, 10^2 PFU; lane 5, 10PFU; lane 6, 1 PFU; lane 7, 10^{-1} PFU; lane 8, 10^{-2}PFU; lane 9, 10^{-3}PFU; lane 10, 10^{-4}PFU; lane 11, 10^{-5}PFU; lane 12, 10^{-6}PFU; lane 13, 10^{-7}PFU.



圖二、以西方墨點轉漬分析之結果。第1行至第9行分別是：單源抗體24D1, 42C, 59H, 212D4, 57A, 210D14, 212D6, 29F, 25C11.。

Fig. 2. Western blotting analysis of selected monoclonal antibodies. From lane 1 to lane 9: monoclonal antibodies 24D1, 42C, 59H, 212D4, 57A, 210D14, 212D6, 7 9F, 25C11.



圖三、以免疫沈澱法分析IBR感染牛及疫苗接種牛血清中之抗體，與標示有L- [^{35}S] methionine之蛋白的反應情形。第1行是未感染之細胞，第2行至第5行分別為病毒感染後0、21、25、及28天之血清；第6行至第8行分別為第二次免疫後0、13、及40天之血清，亦即為第一次免疫後14、27、及54天之血清。

Fig. 3. L-[^{35}S]methionine-labeled polypeptides immunoprecipitated from IBRV-infected cells by sera from infected and vaccinated calves. Lane 1: mock-infected cells; lanes 2 to 5: 0, 21, 25, and 28 days post-infection; lanes 6 to 8: 0, 13, and 40 days after second vaccination, or 14, 27, and 54 days after first vaccination.

一、計劃名稱：

中文：乳鐵蛋白基因轉殖豬之研究發展

英文：Development of lactoferrin transgenic pigs

二、執行期限：1989年7月1日至 1992年6月30日

三、執行單位：臺灣養豬科學研究所

四、美方合作單位及人員：

Dr. Vernon G. Pursel, 美國農業部

五、中方研究人員：朱瑞民、翁仲男、李坤雄、王仕蓉、吳信志

六、計劃目的：

乳鐵蛋白最早是在牛乳中發現的一種攜鐵醣蛋白，分子量約75,000- 80,000daltons。它和血清中之Transferrin，ovatransferrin及menalotransferrin 等皆屬於Transferrin family具高度同源性。至今陸續有人在許多外泌液中發現它的存在，如唾液、淚液、汗液、精漿等，但是仍以乳腺、嗜中性白血球細胞之分泌顆粒含量最高。已知乳鐵蛋白參與多項生物功能，包括抑制細菌生長及負責鐵離子之運送和吸收，促進細胞生長，參與免疫及發炎反應，及影響其他基因之表現等。是否種間或組織間具有不同的調節機制，目前尚不十分清楚。針對豬乳鐵蛋白之研究至今更是有限；然而畜產界中仔豬下痢和貧血始終是普遍性的問題。故本計畫之目的擬先了解豬乳鐵蛋白之生化特性及基因表現機轉，以建立其基礎研究資料並研討解決仔豬下痢和貧血之可行途徑。並配合基因轉殖動物之培育，希望能以改良品種之方式提高育成率。

七、執行成果摘要：

我們建立了 Heparin-Sepharose chromatography 及HPLC(SP-5PW) 系統，自豬乳中純化出豬乳鐵蛋白，以SDS-PAGE 電泳分析結果，顯示其分子量約78,000 dalton，PI值約9.0，和人、牛乳鐵蛋白相同。以鐵離子結合反應及淋巴細胞增生試驗建立豬乳鐵蛋白之定性分析法且證明其與人、牛乳鐵蛋白具相似特性。以純化之豬乳鐵蛋白免疫注射於兔子以製備高力價、高專一性之抗體。經過Protein A column 之純化，收集兔抗豬乳鐵蛋白IgG 抗體配合上Enzyme immunoassay 建立豬乳中乳鐵蛋白之定量測定法。目前亦應用作為其他體液，如血清，精漿等中乳鐵蛋白之分析。

豬乳鐵蛋白 cDNA之篩選方面，我們製備了豬乳腺cDNA 基因庫，依照基因庫中已發表之乳鐵蛋白核酸序列設計primes，先進行PCR(polymerase chain reaction)反應，再以此產物為探針篩選基因庫。經過三次篩選及適當剪接，得到完整之豬乳鐵蛋白cDNA(pBSpLF)，全長約2.3Kb，經過核酸序列分析：5'端具有ATG translation start，3'錯誤! 尚未定義書籤。端具有AATAAA及poly A tail (A15) stop signal。我們所分離之 pBSpLF 和Genebank m61828 (1992, Teng, CT) 序列作比較，結果發現核酸部份二者有99.6% 之相似性，蛋白質部份有4個氨基酸序列差異。而和另一Genebank m92089 (Conneely, OM, 1992) 序列作比較，結果是核酸部份二者有99.4%之相似性，蛋白質部

份有5個氨基酸序列差異。但是這些差異都不是鐵離子結合位置，因此推測可能是族群中個體差異而並不影響其生物功能。我們以限制酵素水解試驗，經電泳分析後，結果和預期圖譜一致，更證明此序列之正確性。我們更進一步以轉錄/轉譯反應來證明此序列可合成出豬乳鐵蛋白，免疫沉澱試驗結果發現此序列所合成之蛋白質分子和豬乳鐵蛋白具相同分子量及抗原性。

小鼠WAP promoter 得自於 Dr.McNight 之實驗室。經過剪接測試後和我們分離之豬乳鐵蛋白cDNA clone進行接合以構築pLF-WAP轉殖基因。然後以顯微注射法注入胚胎細胞以期孕育出pLF-WAP轉殖動物。

八、英文摘要：

We purified porcine whey lactoferrin by affinity chromatography on a heparin-sepharose column, followed by high performance liquid chromatography. Molecular mass of purified lactoferrin(pLF) is 78,000 daltons. The iron- binding activity of pLF had a UV /VIS absorption spectrum indistinguishable from that of human and bovine lactoferrins (absorbamce ratio 465nm/280nm approx 0.046). The growth ratio of WIL-2 cells in pLF -supplemented medium is 70% of that in serum-containing medium. The aforementioned characteristics are similar to those of human and bovine lactoferrins. Immunoblot analysis, using polyclonal antibody raised in rabbits against porcine whey lactoferrin, revealed high specificity for pLF, and low cross reactivity with commercial human and bovine lactoferrins. In addition, we developed an enzyme immunoassay (EIA) to detect lactoferrin in porcine milk. This method appears to have potential to serve as a valuable technique in the mass screening of porcine milk or serum lactoferrin.

We isolated a porcine lactoferrin (LTF) cDNA from sow's mammary gland cDNA library. The nucleotide sequence of this porcine LTF gene is homologous to porcine transferrin (67%), bovine lactoferrin (80%), goat lactoferrin (80%), human lactoferrin (77%), and murine lactoferrin (71%). The porcine LTF sequence shows some mismatches with the previously published sequences M92089 and M81327. Porcine LTF gene and all known lactoferrin and transferrin genes contain the conserved iron-binding and anion-binding sites. Iron and anion binding sites are thus essential for the protein to function. Southern blot hybridization data indicate that porcine LTF gene is a single copy gene in porcine genome as it is in mice and humans. The sequence differences observed among the three porcine LTF genes may represent intra-species genetic diversity.

The deduced amino acid sequence of porcine LTF contains a signal peptide of 19 residues and a mature protein of 685 amino acids. The porcine LTF gene carries 257 bases of 3'-untranslated region (UTR), along with the polyadenylation signal at nucleotide pos-

ition 2273-2278 and polyadenylation tract (A15) at nucleotide 2308. Porcine LTF cDNA when induced to transcribe and translate in vitro, produced a 78 kDa protein that is recognized by rabbit anti-porcine lactoferrin polyclonal antibody. Addition of pure porcine lactoferrin in the in vitro translation system eliminated this specific binding. This competition experiment confirms the identity of PIGLTF as porcine lactoferrin. Furthermore, we has produced some transgenic mice that carried pLF-WAP transgene.

九、已發表報告目錄:

1. Redman M. Chu, Shih-Rong Wang, Chung-Nan Weng and Vernon G Pursel, 1993. Isolation and characterization of porcine milk lactoferrin. American Journal of Veterinary Research, 53(7):1154-1159.
2. Wang Shih-Rong, Chung Nan Weng and Redman M. Chu. 1992. Enzyme immunoassay of porcine milk lactoferrin. Journal of the Chinese Society of Veterinary Science, 18 (4):209-216.

一、計畫名稱：

中文：長期食用含低劑量黃麴毒素飼料對豬隻免疫功能、生長性能以及血液學、血液化學及形態學之影響

英文：Effects of long term consumption of low-level aflatoxin on the immune function, general performance, profiles of hematology and blood chemistry, and morphology of swine

二、執行期限：自1989年7月1日至1992年6月30日

三、執行單位：臺灣養豬科學研究所

四、美方合作單位及人員：

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五、中方研究人員：龐飛、吳繼芳、翁仲男

六、計畫目的：

以活體外和活體內方式探討黃麴毒素對豬隻免疫系統、生長性能以及血液學、血液化學及形態學的影響

七、執行成果摘要：

台灣的溫、溼度均極適合黃麴菌的生長和黃麴毒素的產生，定期的飼料檢測雖可防止高濃度污染的急性中毒症發生，但低量污染仍時有所聞。黃麴毒素曾被指出具干擾免疫系統的作用。疾病的控制是改進豬隻飼養效率中重要的一環，而疾病的發生和免疫系統的完整性有密切的關係，因此是否長期食用含低劑量黃麴毒素飼料對豬隻免疫系統和疾病的發生有不良影響即成為一件值得探討的問題。有關黃麴毒素對家畜禽免疫功能影響之研究多以雞為主，而豬的研究則較少，本研究的目的即藉一系列活體外和活體內的毒性測試，探討黃麴毒素對豬隻淋巴球、肺泡巨噬細胞、整體免疫系統及其它性能的影響。

於活體外，將$5x10^{-2} \times 10^{4}$ ng/ml的黃麴毒素B_1 (AFB_1)與豬血液淋巴球(LY)或肺泡巨噬細胞(AM)混合培養後，分別測定LY的存活率、DNA、RNA及蛋白質合成能力以及白血球中介質II (IL-2)產生能力；AM的存活率、超顯微結構、噬菌力、DNA、RNA及蛋白質的合成能力以及活化作用。LY的結果顯示，$AFB_1 \geq 5 \times 10^1$ ng/ml可導致存活率下降，此與毒素量及作用時間成正相關；DNA和RNA在$\geq 5 \times 10^3$ ng/ml下

呈抑制，而蛋白質和IL-2僅在5×10^4 ng/ml始被抑制。AFB_1無論是對AM的形態或功能，均有不等程度的不良影響，其中以形態、噬菌力及蛋白質合成最為敏感，細胞存活率及DNA和RNA合成次之，細胞活化影響最少；電顯發現偽足收縮和減少，細胞表面出現泡狀突出小體，內質網、粒線體及核週圍小池腫脹，胞質空泡化，最終細胞溶解；噬菌力在不等濃度下減低達10～80%，減低度和濃度成正相關；存活率在5×10^4 ng/ml下6個小時即呈下降，並隨時間延長更趨明顯，在≧5 ng/ml下54小時細胞明顯死亡；DNA、RNA及蛋白質的合成均呈抑制，以蛋白質最敏感DNA次之RNA影響最小；蛋白質和DNA在≧5 ng/ml作用24小時即被抑制，蛋白質抑制在48小時更顯著，DNA則減緩；RNA在5×10^4 ng/ml作用24小時或≧5×10^2 ng/ml作用48小時始抑制。活化作用在≧5×10^2 ng/ml作用24小時或5×10^4 ng/ml作用48小時呈下降。

將含0、25、50、100或200 ppb AFB_1的飼料餵飼4～5週齡豬隻12或20週；12週組中200 ppb小組的平均增重及下顎淋巴結和脾臟重量均較低；AM噬菌力在12或20週組均較差，特別是100和200 ppb兩組，且20週較12週顯著；抗綿羊紅血球之血球凝集抗體力價；血液LY的DNA、RNA、蛋白質及IL-2合成，AM的DNA、RNA及蛋白質合成及活化作用，無論是在12或20週測試組差異多不顯著，但似乎在25和50 ppb兩組多較偏高，在100和200 ppb兩組則多較偏低；血液學、血液化學及形態學則無差異。上述結果顯示，豬隻長期攝食含黃麴毒素100～200 ppb 的飼料，可導致肺泡巨噬細胞功能的減退、淋巴臟器的萎縮及增重下降，但對其它免疫功能和一般表現影響不大。

由於實驗結果顯示豬隻巨噬細胞對黃麴毒素的感受性遠較淋巴球為高，我們推測因巨噬細胞受損進而導致相關的體液和細胞免疫力的減退，可能在黃麴毒素所導致的免疫力下降中扮演著某種角色。肺泡巨噬細胞功能的完整與否和肺炎的產生有著密切的關連性，本研究結果顯示長期餵飼含 100～200 ppb 黃麴毒素的飼料，可導致豬隻肺泡巨噬細胞功能的減退，然此是否即能造成豬隻肺炎產生率的提升，仍有待進一步的探討。

八、英文摘要：

The temperature and humidity in Taiwan favor the growth of *Aspergillus* sp. and aflatoxin production. Although acute aflatoxicosis due to high-level contamination could be controlled by periodic feed screening, unrecognized or ignored low-level contamination is believed to occur in the field. Disease control is one of the major factors affecting the efficiency of pork production. Disease outbreak is closely associated with the integrity of immunity and some acute and subacute studies have shown that aflatoxin may cause immunosuppression. Thus, whether long term consumption of low-level aflatoxin will cause adverse effects on the immune system with subsequent development

of diseases becomes an interesting and essential question to be answered.

The present study consisted of the in vitro and in vivo parts. In the in vitro portion, we investigated the toxicity of aflatoxin B_1 (AFB_1) on swine LY and AM by incubating the cells with $5x10^{-2}$ to $5x10^4$ ng/ml of AFB_1 for various time periods. For LY the parameters examined included cell viability; DNA, RNA, and protein synthesis; and interleukin 2 (IL-2) production. It was found that AFB1 caused significant reductions in the viability of swine LY at concentrations $\geqq 5$ ng/ml and this effect was dose and time -dependent. The DNA, RNA, and protein synthesis and IL-2 production were also inhibited. Statistically significant reductions in DNA and RNA synthesis were seen at concentrations $\geqq 5x10^3$ ng/ml, but only seen in protein synthesis and IL-2 production at the concentration of $5x10^4$ ng/ml. For AM the parameters examined included ultrastructure (SEM & TEM); viability; phagocytosis potential; DNA, RNA, and protein synthesis; and cell activation. The data showed that the ultrastructure, phagocytosis potential, and protein synthesis were the most sensitive followed by viability and DNA and RNA synthesis with cell activation affected the least. Both SEM and TEM demonstrated contraction, bleb formation, and reduction in the number of pseudopodia at concentrations $\geqq 5x10^4$ ng/ml and cell lysis at the highest concentration. The potential of AFB_1-treated AM uptaking *Staphylococcus aureus* was reduced 10～80% in a dose-dependent manner as compared to that of control. DNA, RNA, and protein synthesis were all markedly reduced with protein synthesis affected the most. Protein and DNA synthesis were significantly reduced at concentrations $\geqq 5$ ng/ml by 24 hours; by 48 hours, the reduction became more obvious in the former but less evident in the latter. Significant reduction in RNA synthesis was noted at the concentration of $5x10^4$ ng/ml by 24 hours or at concentrations $\geqq 5x10^2$ ng/ml by 48 hours. The activation of AM by lipopolysaccharide (LPS) was decreased at concentrations $\geqq 5x10^2$ ng/ml by 24 hours or $\geqq 5x10^4$ ng/ml by 48 hours.

In the in vivo portion, two animal studies were conducted. The effects of aflatoxin on the immune function, general performance, profiles of hematology and blood chemistry, and morphology of swine were evaluated following consumption of feed containing 0, 25, 50, 100, or 200 ppb of AFB_1 (toxic potency) for 12 and 20 weeks. There was a dose -related weight reduction in both spleen and right submandibular lymph node of the 12-week group and a significant difference was seen in the spleen between the 200 ppb group and control; however, this was not apparent in the 20-week group. Similar changes were also noted in the body weight gain. The humoral immune response to sheep red blood

cells, measured by hemagglutination titer, revealed compatible or enhanced responses in AFB_1-treated groups as compared with control. The DNA, RNA, and protein synthesis as well as interleukin 2 production of concanavalin A-stimulated LY in the AFB_1-treated pigs of either 12 or 20-week group were enhanced, although generally not statistically significant. The phagocytosis potential of AM was reduced in the AFB_1-treated pigs of both 12 and 20-week groups, especially at the doses of 100 and 200 ppb; the level of reduction was increased as the duration of exposure increased from 12 to 20 weeks. For the DNA, RNA, and protein synthesis, and LPS-induced cell activation of AM, the lower doses (25 and 50 ppb) had a tendency to enhance the response whereas the higher doses (100 and 200 ppb) had a tendency to reduce the response. No alterations were revealed in blood chemistry, blood counts, and morphology of major organs. The result indicates long term consumption of aflatoxin- contaminated feed at a level of 100 ~ 200 ppb in swine can cause adverse effects on various aspects of AM, lymphoid organ weight, and body weight gain but not in other immune functions and general performance.

In conclusion, the present study has demonstrated that in swine macrophages are more susceptible to aflatoxin than are lymphocytes both in vitro and in vivo. It is speculated that altered macrophage-dependent immunocompetence may play an important role in the aflatoxin-related reduction in immune function, particularly when aflatoxin exposure level is low. However, whether this macrophage-associated immune-compromise will predispose the affected animal to infection remaines to be answered.

九、已發表報告目錄：

1. Pang, V. F., Chang, C. C., Weng, C. N., and Wu, J. F. 1992. The in vitro toxicity of aflatoxin B_1 on swine alveolar macrophages. Proceedings of International Pig Veterinary Society 12:668.
2. Pang, V. F. and Pan, C. Y. 1994. The cytotoxic effects of aflatoxin B_1 on swinelymphocytes in vitro. J. Chin. Soc. Vet. Sci. 20:289-301.

一、計畫名稱：

中文：豬血清中脂肪細胞分化抑制蛋白之分離及其在養豬上之應用

英文： Isolation a pig serum adipocyte differentiation inhibitor and its application in swine industry

二、執行期限：1995年7月1日 至 1998年6月30日, 共3年

三、執行單位：台灣養豬科學研究所

四、美方合作單位及人員：

Dept. of Animal Science, Oregon State University

Dr. Ching-Yuan Hu

五、中方研究人員：劉昌宇、張玲菊

六、計畫目的：

分離豬血清中脂肪細胞分化抑制蛋白，瞭解抑制蛋白作用機制。並篩選其基因以便大量製造後，將之應用在養豬事業，以達到抑制體脂肪蓄積、改善肌肉生長與飼料效率的目的。

七、執行成果摘要：

肉豬上市時，脂肪組織佔屠體重量的25 %或更高。過多的體脂肪蓄積，表示飼料能量的浪費、生產成本的增加；控制體脂肪生長，使原本用於脂肪合成的能量轉為蛋白質合成，可促進瘦肉生長、改善飼料利用效率。過去的研究顯示，血液中存有不明因子，能抑制豬脂肪細胞分化、脂質蓄積。本試驗之目的即在純化豬血清中脂肪細胞分化抑制因子，瞭解抑制作用機制，進而應用在養豬生產，以減少脂肪組織、改善瘦肉生長與飼料效率。取1～3日齡藍瑞斯仔豬背部脂肪組織，經collagenase分解、離心、過濾，收集脂肪先驅細胞 (preadipocytes)。 脂肪先驅細胞 (5 x 10^4 cells /cm^2) 在含有2 %血清之DME/F12培養液中，幾乎無細胞分化的現象 (以glycerol-3-phosphate dehydrogenase, GPDH酵素活性表示)。改以含insulin (500 μ g/ml)，triiodo-thyronine (2 ng/ml)，transferrin (50 μ g/ml) 和hydrocortisone (50 μ g/ml) 培養液 (ITTH) 培養，GPDH活性在10 天後提高6倍 (106 vs 641 nmoles/min/mg protein, P<0.01)。利用親合性層析管柱純化血清因子，所得蛋白質液在ITTH培養液中可抑制50 % GPDH酵素活性 (P<0.05)，顯示抑制因子的存在。因抑制因子經加熱處理 (80℃) 或trypsin作用後，其抑制脂肪細胞分化的能力減退或喪失，所以此抑制因子應屬蛋白質類物質。目前試驗正繼續純化脂肪細胞抑制蛋白，希望能得到單一蛋白，以便用來製造抗體、篩選基因，進而瞭解抑制蛋白構造。純化的抑制蛋白將用來處理豬隻，以降低體脂肪生長。

八、英文摘要

When hogs reach market weight, the percentage of body fat is over 25 % of the

carcass. Excess body fat indicates waste of dietary energy and increase of production cost. Producing pigs with less fat deposition would shift energy from lipid synthesis to protein synthesis, resulting in improvements in lean gain and feed efficiency. Previous studies have indicated that unknown serum-born factors inhibit porcine adipocyte differentiation and lipid accumulation in vitro. Therefore, the objectives of this study are to isolate and characterize the serum adipocyte 'differentiation inhibitor and to apply the inhibitor to swine production. New born Landrace piglets between 1 and 3 days of age were killed by CO_2 asphyxiation, and dorsal subcutaneous adipose tissue was aseptically removed. Preadipocytes were obtained by collagenase digestion and seeded in DME/F12 medium at a density of 5 x 10^4 cells/cm^2 at 37℃ in 95 % air/5 % CO_2. When 2 % serum was added to the medium, little cell differentiation was detected as determined by glycerol-3-phosphate dehydrogenase (GPDH) activity. In contrast, the combination of insulin (500 ng/ml), triiodothyronine (2 ng/ml), transferrin (50 μ g/ml), and hydrocortisone (50 ng/ml) (ITTH medium) significantly ($P<0.01$) stimulated GPDH activity after 10 nmoles /min/mg protein). Serum inhibitor purification was performed with Affi-Gel Blue chromatography, and the effluent decreased 50 % of GPDH activity in ITTH ($P<0.05$) as compared with no effluent supplemented culture. Since the inhibitor was heat (80℃) and trypsin sensitive, in which the inhibitory effect on adipocyte differentiation was abolished, the inhibitor appeared to be a protein- like factor. Current studies are trying to further purity the adipocyte differentiation inhibitor and to apply purified protein to swine production.

一、計畫名稱：

中文：台灣牛小型焦蟲之疫學研究及防疫

英文：Epizoological and preventive study of Theileriosis in Taiwan

二、執行期限：1988年7月1日至1992年6月1日

三、執行單位：國立台灣大學獸醫學系

四、美方合作單位及人員：University of Illinois, Dean Richard Derks

五、中方研究人員：張政宏、費昌勇

六、計畫目的：

於亞熱帶地區之台灣，吸血昆蟲及壁蝨全年存在，為主要血液寄生蟲之傳播病媒。牛之大、小型焦蟲及邊蟲經常出現，但出現率病媒及種類等流行病資料不明瞭，本計畫是確定其流行病學現況。

七、執行成果摘要：

根據電子顯微鏡切片觀察感染小型焦蟲之紅血球所見之bar structure得知本省所存在者為 *Theileria sergenti*。此外,本報告亦以補體結合反應探討本省大型焦蟲(*Babesia bigemina*)，邊蟲(*Anaplasma marginale*)，及前述之小型焦蟲三者在牛群中之血清學陽性比率。並比較三者在品種、地區、性別及年齡之差異性。結果顯示總陽性率在邊蟲、大型焦蟲及小型焦蟲分別為33.7%, 29.2%,及23.7%；三者在品種,地區,性別,及年齡均無顯著差異。

八、英文摘要：

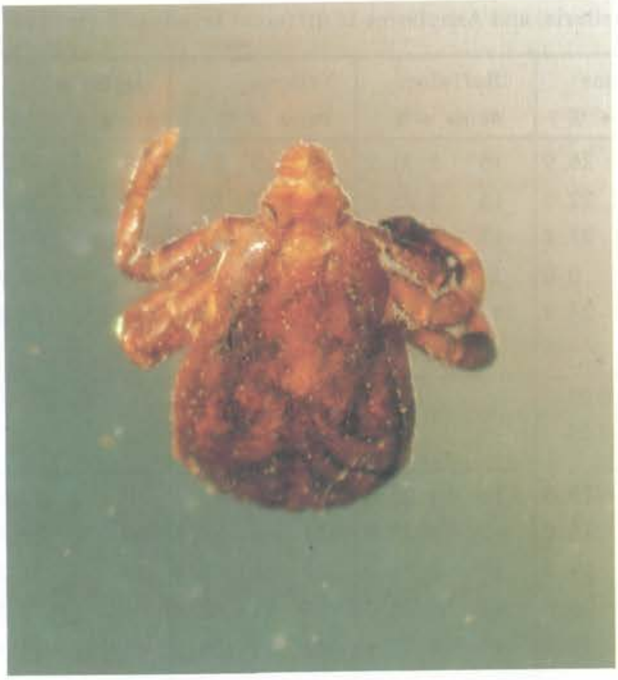
Theileria sergenti was isolated and identified by transmission electron microscope, based on its characteristic "bar-structure". Seroprevalance of complement fixing antibodies specific to the organisms of *Babesia bigemina, Anaplasma marginale,* and *T. sergenti,* was also investigated. Totally 308 bovine serum samples collected from dairy cattle across Taiwan were tested. The data was analyzed with respect to the breed, sex, and age of the animals and geographic location (north, central, south, and east). The cumulative frequencies were 29.2%, 33.7%, and 23.7% for babesiosis, anaplasmosis, and theileriosis, respectively. There was no significant ($P>0.05$) differences due to age, sex, breed of animals, or geographic location for each of the diseases.

九、已發表報告

Fei, Andrew Chang-Young. 1991. Epizoological study of Theileriosis, Babesiosis, and Anaplasmosis in Taiwan. Memoirs College Agriculture National Taiwan University 31 (3):14- 27.

Prevalence of Babesia, Theileria, and Anaplasma in different breeds and areas of Taiwan cattle

Areas	Holsteins			Buffaloes			Yellows			Hybrids			Total		
	#cow	+	%	#cow	+	%	#cow	+	%	#cow	+	%	#cow	+	%
B. bigemina	26	7	26.9	16	5	31.2	9	3	33.3	9	3	33.3	60	18	30.0
North	50	11	22.0	12	3	25.0	21	7	33.3	20	6	30.0	103	27	26.2
Middle	61	17	27.8	17	5	29.4	16	4	25.0	16	7	43.7	220	33	30.0
South	4	0	0.0	20	9	45.0	7	2	28.5	4	1	25.0	35	12	34.2
East	141	35	24.8	141	22	33.8	141	16	30.1	49	17	34.6	308	90	29.2
Total															
Breeds: $x2=2.72$	d.f.=3 $p>0.05$														
Areas: $x2=0.93$	d.f.=3 $p>0.05$														
T. sergenti	26	4	15.3	16	4	25.0	9	2	22.2	9	2	22.2	60	12	20.0
North	50	8	16.0	12	3	25.0	21	6	28.5	20	4	20.0	103	21	20.3
Middle	61	15	24.5	17	6	35.2	16	4	25.0	16	5	31.2	220	30	27.7
South	4	0	25.0	20	5	25.0	7	3	42.8	4	1	25.0	35	10	28.5
East	141	28	19.8	141	18	27.6	141	15	28.3	49	12	24.4	308	73	23.7
Total															
Breeds: $x2=2.36$	d.f.=3 $p>0.05$														
Areas: $x2=2.31$	d.f.=3 $p>0.05$														
A. marginale	26	9	34.6	16	7	43.7	9	3	33.3	9	4	44.4	60	23	38.3
North	50	17	34.0	12	1	8.3	21	7	33.3	20	7	35.0	103	32	31.0
Middle	61	19	31.1	17	8	47.0	16	5	31.2	16	6	37.5	220	38	34.5
South	4	0	50.0	20	6	30.0	7	0	0.0	4	3	75.0	35	11	31.4
East	141	47	33.3	141	22	33.8	141	15	28.3	49	20	40.8	308	104	33.7
Total															
Breeds: $x2=1.81$	d.f.=3 $p>0.05$														
Areas: $x2=1.01$	d.f.=3 $p>0.05$														



圖一、小型焦蟲之病媒壁蝨 *Haemaphysalis* spp.。

Fig. 1. Tick vector of Theileriosis in Taiwan — *Haemaphysalis* spp.

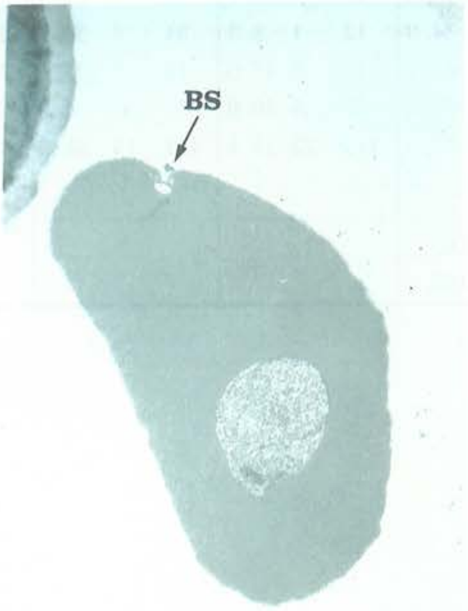


Fig. 2. Transmission electron microscopy of *Theileria sergenti* in infected RBC.
Arrow indicated the bar-structure of organism × 16,000.

一、計畫名稱：

中文：牛指狀絲蟲在台灣之流行病學及防疫研究

英文：Epizoological and preventive study of *Setaria digitata* in Taiwan

二、執行期限：1993年7月1日至1996年6月1日

三、執行單位：國立台灣大學獸醫學系

四、美方合作單位及人員：

University of Illinois, Dean Rechard Dierks

五、中方研究人員：費昌勇教授、林大盛副教授

六、計畫目的：

指狀絲蟲(*Setaria digitata*)是東方特有之牛寄生蟲，寄生於牛之腹腔。最近十年來，台灣有甚多鹿之死亡病例於其腦中發現類似本蟲之幼蟲。筆者認為可能原因是當本蟲之幼蟲於非固有宿主體內週轉經過腦部時造成腦之致害。故本蟲為本省現階段防疫之重要課題。鑒於本蟲在世界上之分佈稀少，相關性研究報告在國內外均不多，且由於本省為該蟲之疫區，台灣四面環海的地理環境亦適合本蟲之研究，故本計畫無論在經濟抑或學術方面，均有價值。

七、執行成果摘要：

本研究在了解本蟲在台灣之疫學狀況並試圖建立有效之偵測方法。內容包括: 1)調查本蟲在牛鹿之感染狀況；2)找出本蟲之主要抗原；3)研究其粒腺體DNA ITS-2基因序列冀從其序列得到診斷方法。在目標1)之研究中已解剖585隻奶牛及422隻鹿，分佈呈全省性，病理解剖發現腹腔會長出結節，內含蟲體有不同程度之鈣化現象。在目標2)之研究中共純化了4種抗原，分別是蟲體抗原、不溶性抗原、分泌抗原、及表面抗原。這4種抗原中均顯示60.5KD及54.3KD有反應。在目標3)之研究中得知 *Setaria digitata* ITS-2之核酸序列有245個base， 與 *Setaria marshalli* 有96%之相似性。結論：可找出對 *Setaria* spp.之特異性診斷引子。

八、英文摘要：

The purposes of this study are to understand the epizoological pattern of Setariasis in Taiwan and attempting to develop an effective measurement to control the infection. Study contains: a) to survey and study the pathology of *Setaria spp.* infestation in Taiwan sicadeer and cattle; b) to find the major antigens of *S. digitata* which can induce immune response in natural hosts; c) to study the ribosomal DNA ITS-2 sequence of the bovine parasite *Setaria* spp. to find a genetic method of diagnosis. In the first topic, we collected and identified the parasites from the peritoneal cavity of necropsied Holstein cows and Taiwan scicadeer (*Cervus taionanus*) of various ages during 1991-1993. In this study, totally one hundred and fifty nine out of 585 cows from 11 prefectures and 18 out of 422

sicadeer from 6 prefectures had adult worms in the peritoneal cavities. The distribution of *Setaria* spp. appeared to be islandwide in Taiwan, although there were no data available in few prefectures. Pathologically, nodules with *Setaria* spp. were found in the peritoneum of some of the cows examined. The worms were calcified in different degrees. The pathological changes of these nodules were described in detail in the first year report. In the second topic, 4 separate types of antigens were purified: a) Soluble antigen obtained fom the whole worm body; b) Insoluble antigen treated with detergent; c) Excretory/Secretory (ES) antigen obtained from the parasite's culture media; d) Surface antigen purified with nOG treatment. These 4 antigens are then compared running SDS-PAGE electrophoresis. After using silver stain or western blotting, no matter which antigen showed two prominent bands at 60.5KD and 54.3KD. (results of the second year report). In the third topic, we analyzed the ITS-2 sequence of *Setaria digitata* and found 245 base pairs. Compared with the ITS-2 sequence of *Setaria marchalli,* the similarity is 96% (results of the third year report, included in this final report). In conclusion, it is predictable that a primer will be developed in the near future for the diagnosis of bovine *Setariasis.*

九、已發表報告：

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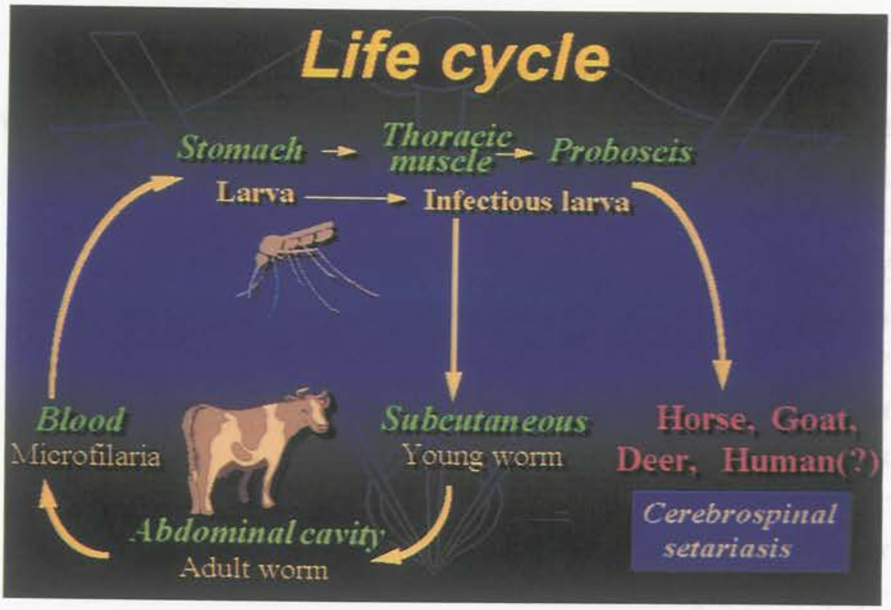
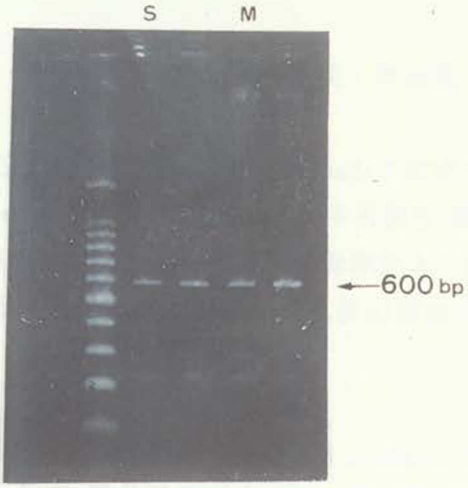


圖一、指狀絲蟲之生活史

Fig. 1. Life cycle of *Setaria digitata*.



圖二、蟲體所有之 DNA 經由NC2，NC4 引子增幅之 PCR 產物。

S=*Setaria digitata*，M=*Setaria marshalli*

Fig. 2. PCR products of total worm DNA amplified by primers NC2 and NC4.

一、計畫名稱：

中文：純種及雜交種肉牛遺傳與環境相關之研究

英文：Long term purebred and crossbred cattle genetic and management study

二、執行期限：1987年7月1日至1997年6月30日，共10年。

三、執行單位：臺灣省畜產試驗所恒春分所

四、美方合作單位及人員：

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六、計畫目的：

利用恒春分所現有之聖達牛(Santa Gertrudis)與國外引進之耐熱肉牛品種-布拉曼牛(Brahman)以及溫帶肉牛品種-德國黃牛(Gelbvieh)與夏洛利牛(Charolais)，進行純種繁殖及雜交配種試驗，期能產生適合本省環境且又具備優良之肉牛重要經濟性狀如生長迅速、繁殖性能佳、產肉能力高好及屠體值高之肉牛品種，推廣農民以改善本省肉牛體質，提升自產牛肉之競爭力。

七、執行成果摘要：

前五年(1987的第一階段(phase 1)之中，以聖達牛(SG)及布拉曼牛(BR)進行純種繁殖，並與德國黃牛(GV)進行雜交配種，所生產的四個品種-純種聖達(SG)、純種布拉曼(BR)、德國黃牛與聖達雜交種(GS)、德國黃牛與布拉曼雜種(GB)，以(GB)表現較好－有較佳的生長能力(公牛一歲齡體重可達348kg，平均日增重1.27kg)、較高的屠體精肉率(65.7%)與屠體價值(較其他品種售價每頭多2,768～4,197，且環境適應能力良好(與耐熱之布拉曼牛相近)。另外，雜種牛的表現比純種牛好，顯示雜交優勢得到改良效果。後五年(1992的第二階段(phase 2)中，以夏洛利牛(Charolais)作為terminal sire，與第一階段的四

個品種母牛進行二及三品種雜交，亦生產四個品種－夏洛利與聖達雜交種(CS)、夏洛利與布拉曼雜交種(CB)、夏洛利×德國黃牛×聖達三品種雜交(CGS)及夏洛利×德國黃牛×布拉曼三品種雜交(CGB)，目前試驗仍進行中，尚無具體性之完整結果。已累積所得的結果顯示：出生體重因受環境影響小，故能表現出遺傳影響之效果，即體重大小依所含溫帶牛血液比例之多寡而依次漸輕－CGS(90.625%)>CS (81.25%)>CGB(75%)>CB(50%)。仔牛於哺乳期間隨母牛放牧，環境較粗放，故增重及離乳體重以含耐粗熱之布拉曼血統的雜交種，表現比含聖達血統的雜交種為佳。離乳至一歲階段因環境改為圈飼，遺傳潛能得以發揮，三品種雜交有表現較佳的趨勢。

八、英文摘要：

Santa Gertrudis and Brahman cattle were straightbred and crossbred with Gelvieh sires during the phase 1 period (1987～1992). straight Santa Gertrudis (SG), straight Brahman (BR), Gelbvieh × Santa Gertrudis crossbred (GS) and Gelbvieh × Brahman crossbred (GB), the GB had better growth performances (average yearling weight and postweaning ADG of bulls are 348 kg and 1.27 kg), higher lean/carcass ratio (65.7%) and carcass retail prices (NT$2,768 local environment well (similar to Brahman). Crossbred cattle performed better than the straightbred cattle due to the effect of hybrid vigor. During the phase 2 period (1992～1997), Charolais were mated as the terminal sire with the cows that produced in the phase 1 period . Four breed types were thus produced － Charolais × Santa Gertrudis crossbred (CS), Charolais × Brahman crossbred (CB), Charolais × Gelbvieh × Santan Gertrudis crossbred (CGS) and Charolais × Gelbvieh × Brahman crossbred (CGB). Data are still under collecting and the present results indicate ： birth weights were affected by the percentages of *Bos taurus* blood － CGS(90.625%)>CS(81.25%)>CGB(75%)>CB(50%). Calves were with their mothers on pastures during the suckling period. Calves with Brahman blood (roughage and heat tolerant) performed better than those with Santa Gertrudis blood in average daily agins (ADG) and weaning weights. The three-breed crosses (CGS and CGB) tended to perform better due to the genetic potential (maximum hybrid vigor) as environment improved to feedlot during the period from weaning to yearling.

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附錄二：1986-1995 中美農業科學合作計畫名稱

Appendix II: Project titles of COA-USDA agricultural science cooperative program (1986-1995)

第一期(1986)

計畫名稱	執行機構	主持人
1. 菌根菌之接種與礦區廢土石地之復舊造林 Mycorrhizae and establishment of trees on mine soils	台灣省林業試驗所	鍾旭和
2. 台灣家禽流行性感冒監測 Avian influenza surveillance in Taiwan	中興大學農學院 台灣省家衛所	謝快樂 呂榮修
3. 草蝦生殖生理與成熟控制研究 Reproductive physiology and controlled maturation of tiger prawns, *Penaeus monodon*	台灣大學漁科所	郭欽明
4. 噬菌體誘導(致)柑桔潰瘍病菌遺傳性質之變化及其對致病力之影響 Phage-induced changes in genetic characterics and their effect on virulence and pathogenicity of *Xanthomonas campestris* pv. *citri*	中興大學農學院	吳文川
5. 台灣豬假性狂犬病控制與流行病學研究 Epizootiological survey and prevention of pseudorabies infection in Taiwan	中興大學農學院	王吉德
6. 水車對漁池影響之研究 The effects of paddlewheel on pond dynamics	中山大學	方新疇 方力行
7. 高成長率吳郭魚育種之研究 Selection for growth rate in tilapia	省水試所東港分所	黃齊明

第二期(1987)

計畫名稱	執行機構	主持人
1. 大豆油耐高溫穩定性研究 Improvement of soybean oil at high temperature	食研所	朱燕華
2. 降低果實貯藏寒害之研究 Reduction of chilling injury in fruits	台灣大學農學院	王自存
3. 少量有機添加物防治*Rhizoctonia solani* 苗猝倒病之研究 Biological control of *Rhizoctonia solani* with minimal organic amendment	台灣大學農學院	莊再揚
4. 建立甘藷病毒標準檢定制度 Development of a standard procedure for sweet potato virus indexing	亞蔬中心 中興大學	S. K. Green 徐堯煇
5. 養殖蝦類傳染性病之研究 Studies on the infectious diseases of cultured prawns	台灣大學理學院	郭光雄 陳秀男
6. 養殖草蝦之蛋白質需求與消化研究 Protein requirement and digestion in cultured *Penaeus monodon*	台灣海洋學院	孫寶年
7. 純種及雜交種肉牛遺傳與環境相關性之研究 Long term purebred and crossbred cattle genetic and management study	省畜試所恆春分所	洪嘉謨
8. 豬弓蟲病之研究—疫學、病理學及疫苗開發 Toxoplasmosis in swine epidemiology, pathology, and vaccine development	屏東農專	張甘楠
9. 豬附紅血球立克次體病—診斷、免疫及防治之研究 Eperythrozoonosis in swine- diagnosis, immunology and control	台糖畜研所	徐興鎔
10. 美國木材在台灣之市場調查 Marketing research of imported timber from the United States in Taiwan	省林試所	任憶安

11. 高比重闊葉樹材之加工研究 | 省林試所 | 唐讓雷
Machining and gluing of high density hardwoods

12. 生物快速測定系統發展 | 中興大學農學院 | 曾浩洋
Development of biosensor survey of salmonella contamination in foods, development of DNA probes and comparison of it with the EIA kit for rapid detection of salmonella

13. 輻射線對草蝦超微細結構及細胞化學之影響 | 食研所 | 楊瑞森
Ultrastructural and cytochemical effects of gamma radiation on grass shrimps *Penaeus monodon*

第三期(1988)

計畫名稱	執行機構	主持人
1. 草蝦之品質維護與廢棄物之利用 Keeping the quality of post-mortem grass prawn and utilization of the discards from processing	台灣海洋學院	陳幸臣
2. 吳郭魚耐寒性之生理生化與育種研究 The biomedical analysis and genetics selection of to cold tolerance	台灣海洋學院 台灣省水試所	張清風 湯宏吉
3. 臺灣設施栽培系統之研究開發 Engineering protected cultivation systems in Taiwan	臺灣大學農機系	吳中興
4. 烹調與加工對肉品中殘留農藥及藥物之影響 Influence of cooking/processing conditions on pesticide/drug residues in meat and poultry	台灣大學食科所	許順堯
5. 輻射照射對雞肉之影響 Effects of ionizing radiation on pork, poultry, and prawns	台灣大學食科所	郝龍斌
6. 血清學探針之開發用於柑桔病原之檢驗與標誌探針應用於探測病原感染及傳播 Development of serological probes for detection of citrus pathogens and application of labeled probes for measuring pathogen infection and dispersal	台灣大學農學院	蘇鴻基

7. 傳染性鼻氣管炎自然感染牛與免疫牛鑑別診斷之研究 Differentiation of natural infection from vaccination with inactivated virus of infectious bovine rhinotracheitis in cattle	中興大學農學院	吳福明
8. 臺灣牛小型焦蟲之疫學研究及防疫 Epizoological and preventive study of Theileriosis in Taiwan	臺灣大學農學院	張政宏

第四期(1989)

計畫名稱	執行機構	主持人
1. 台灣作物根部疫病之抑病土壤檢定及抑病機制研究 Detection and mechanisms of soils suppressive to root rot caused by *Phytophthora* species in Taiwan	省農試所嘉義分所	安寶貞
2. 觀賞植物病毒快速檢測及其防治 Rapid method for diagnosing ornamental plant viruses and their control measures	中興大學植病系 臺灣省農業試驗所	柯南靖 張清安
3. 木瓜輪點病毒基因用在轉型木瓜植物之表現 Expression of papaya ringspot virus genes in transgenic papaya	中興大學植病系	葉錫東
4. 養殖青魚死亡原因之研究 Studies on the causes of mortality of cultured black carp, *Mylopharyngodon piceus*	台灣大學理學院 省水試所	陳秀男 廖一久
5. 草蝦桿狀病毒診斷技術開發研究 Molecular biology and biotechnology of monoden baculovirus (MBV)	中研院動物所 屏東農專獸醫科	吳金洌 董明澄
6. Lactoferrin 基因轉殖豬之研究發展 Development of lactoferrin transgenic pigs	臺灣養豬科學研究所	朱瑞民
7. 長期食用含低劑量黃麴毒素飼料對豬隻免疫功能、一般性能、血液和血液化學及組織形態的影響 Effects of long term consumption of low-level aflatoxin on the immune function, general performance, profiles of hematology and blood chemistry, and morphology of swine	臺灣養豬科學研究所	龐　飛 吳繼芳

第五期(1990)

計畫名稱	執行機構	主持人
1. 本省家禽流行性感冒之監測 Avian influenza surveillance in Taiwan	中興大學	謝快樂
2. 黃豆水分含量及油品中微量物質對精練油品質之影響 Effects of minor constituents of soybean oil from soybeans of various moisture content on the quality of RBD soybean oil	食研所	朱燕華
3. 人工種子技術的發展 Dev.elopment of artificial seed technology	生技中心	宋台生
4. 生物合理性化合物在害蟲防治之應用 Development of biorational compounds for insect control	中興大學	侯豐男
5. 牛邊蟲病流行病學、血清與防治之研究 Epidemiology, serology and control of bovine anaplasmosisin Taiwan	省家畜衛生所 台糖畜研所	蘇杰夫 徐興鎔
6. 擠壓加工對米製品物理及化學性質影響之研究 Extrusion cooking of rice a physical and chemical characteristics study	臺灣大學	蔣丙煌
7. 以脫皮全脂黃豆粉全部利用製造黃豆食品 Total utilization of hulled soybean flour for soybean products	食研所	陳文亮

第六期(1991)

計畫名稱	執行機構	主持人
1. 雜交技術之改進及聚合鏈反應應用於食品中沙門氏菌與李斯特菌之檢測 Improving the DNA hybridization technique and application of PCR for rapid detection of Salmonella and Listeria in foods	中興大學	曾浩洋

2. 利用美國櫟樹小徑木栽培香菇之研究 Cultivation of shiitake with small diameter logs of american oaks	林試所	鍾旭和

第七期(1993)

計　畫　名　稱	執　行　機　構	主持人
1. 利用導管侷限性 *Clavibacter xyli* subsp. *cynodontis* 構築植物系統性遺傳特性轉殖系統 Construction of an endophytic genetic delivery system from xylem- limited *Clavibacter xyli* subsp. *cynodontis*	台灣大學	林長平
2. 利用遺傳工程誘導對蘭花喜姆比蘭嵌紋病毒產生抗性之研究 Genetically engineered resistance to cymbidium mosaic virus in orchid	農試所鳳山分所	王惠亮
3. 台灣重要作物Closterovirus 病害之診斷與防治技術研究 Studies on the diagnostic and control techniques of closterovirus diseases among important crops in Taiwan	中興大學	曾德賜
4. 開發蟲生真菌作為防治農業害蟲用微生物殺蟲劑及其應用研究 Formulation and application of eutomopathogenic fungi as mocrobial insecticides for biocontrol of agricultural pests	屏東技術學院	劉顯達
5. 利用分子標誌選育多重抗病毒之熱帶番茄品種之方法研究 Methodology development of multiple virus resistance in tropical tomatoes using molecular markers	亞蔬中心	S. K. Green
6. 天國栗高硫蛋白質基因在毛豆及綠豆的表現 Expression of sulfur-rich protein gene from paradise nut in mungbean and vegetable soybean for nutritional quality improvement	亞蔬中心	宋　達
7. 牛指狀絲蟲在台灣之流行病學及防疫研究 Epizoological and preventive study of *Setaria digitata* in Taiwan	台灣大學	費昌勇

8. 純種及雜交種肉牛遺傳與環境相關之研究 Long term purebred and crossbred cattle genetic and management study	畜試所恒春分所	成游貴
9. 台灣養殖蝦類細菌性疾病之快速診斷及防治研究 Studies on the rapid diagonsis and prevention of bacterial diseases of cultured shrimp in Taiwan	台灣大學	陳秀男
10. 國有人工林永續生態系經營的研究 Development of sustainable management for plantation in national forests	林試所	楊政川
11. 線性規劃及模糊目標規劃在森林經營計畫決策上之應用 An application of linear programming and fuzzy goal programming to the forest management planning and decision-making	台灣大學	楊榮啟

第八期(1994)

計畫名稱	執行機構	主持人
1. 轉移色素合成基因創造新花色之晚香玉及金花石蒜 Developing new flower colors by transformation of pigment biosynthetic gene(s)	台灣省農業試驗所 種苗改良繁殖場 國立中興大學	王強生 陳駿季 宋濟民
2. 台灣薊馬傳播之蕃茄斑萎病毒群之研究 Study on thrips-borne tospoviruses in Taiwan	台灣省農業試驗所 台中區農業改良場 國立中興大學 屏東技術學院	張清安 陳慶忠 葉錫東 邱人璋 陳滄海
3. 構築以豬假性狂犬病病毒為載體之多價疫苗 Construction and characterination of recombinant pseudorabies virus-based multivalent vaccines	國立台灣大學 中央研究院	潘銘正 呂勝春 張清華
4. 開發抗傳染性胰臟壞死病毒核醣轉殖魚類 Development of ribozyme technology for the production of transgenic fish resistant to infectious pancreatic necrosis virus (IPNV)	中央研究院 生技中心	吳金洌 徐亞莉 張繼堯 陳鐵雄

第九期(1995)

計畫名稱	執行機構	主持人
1. 研發對環境安全且有效之住宅與商業用木製品抗燃劑 Development of effective and environmentally safe fire retardant for wood products used in residential and commercial buildings	台灣省林業試驗所	鄒哲宗
2. 豬血清中脂肪細胞分化抑制蛋白之分離及其在養豬上之應用 Isolation a pig serum adipocyte differentiation inhibitor and its application in swine industry	台灣養豬科學研究所	劉昌宇

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